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SYNTHESIS, STRUCTURAL CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF NOVEL PICOLINIC ACID-DERIVED PYRIDINYLAMINO CARBOXAMIDES AS POTENTIAL ANTICANCER AND ANTIBACTERIAL AGENTS

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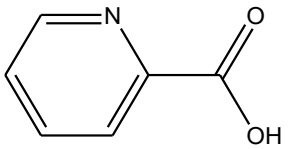
ABSTRACT:
The development of structurally diverse heteroaromatic scaffolds remains an important strategy in the search for bioactive small molecules with anticancer and antimicrobial potential. In the present study, a series of ten N,N-disubstituted-4-(pyridin-2-ylamino) pyridine-2-carboxamide derivatives (IVa–IVj) was synthesized through a sequential transformation of picolinic acid involving formation of 4-chloropyridine-2-carbonyl chloride, amidation, and subsequent nucleophilic substitution with 4-aminopyridine. The synthesized derivatives were obtained in 63–72% yields and characterized using physicochemical and spectroscopic techniques. Their biological potential was investigated by in vitro cytotoxicity assessment against MCF-7, HT-29, and A549 human cancer cell lines using the MTT assay and by antibacterial screening against selected Gram-positive and Gram-negative bacterial strains using the cup-plate method. The derivatives exhibited variable biological activities depending on structural modification. Among the synthesized compounds, IVc demonstrated the most pronounced cytotoxic activity, with IC_{50} values of 9.2, 7.50, and 16.2 μM against MCF-7, HT-29, and A549 cells, respectively. IVh also exhibited strong cytotoxicity, with corresponding IC_{50} values of 12.8, 9.4, and 17.5 μM. Notably, IVc showed the strongest antibacterial activity, producing inhibition zones of 12–15 mm against the tested bacterial strains, while IVh exhibited zones of 10–14 mm. Although the reference agents cisplatin and ciprofloxacin showed greater activity under the respective assay conditions, IVc and IVh demonstrated a favorable overall biological profile. These findings identify the pyridinylamino-picolinamide scaffold, particularly derivatives IVc and IVh, as a promising starting point for further structural optimization and mechanistic investigation.

KEYWORDS: Picolinic acid; pyridine carboxamide; heterocyclic compounds; cytotoxicity; MTT assay; antibacterial activity; structure–activity relationship.

INTRODUCTION

Picolinic acid (pyridine-2-carboxylic acid, Fig. 1) is a structurally versatile heteroaromatic carboxylic acid in which a carboxyl group is positioned adjacent to the ring nitrogen of a pyridine nucleus [1, 2]. The presence of two chemically distinct functional sites within a compact heteroaromatic framework provides considerable scope for molecular modification and the development of biologically active derivatives [3]. Picolinic acid and its derivatives have attracted sustained interest in medicinal and bioorganic chemistry because structural modification of the pyridine ring and carboxamide functionality can influence molecular recognition, physicochemical properties, and biological activity [4]. In particular, the pyridine nitrogen can participate in hydrogen-bonding and coordination interactions, whereas transformation of the carboxylic acid into amide derivatives provides an effective strategy for modulating lipophilicity, hydrogen-bonding capacity, conformational characteristics, and overall pharmacological behavior [5].



Picolinic acid

Fig. 1. Chemical structure of Picolinic acid.

Heteroaromatic carboxamides represent an important class of bioactive molecules because the amide functionality can act as both a hydrogen-bond donor and acceptor and can establish productive interactions with biological macromolecules. Incorporation of substituted amino-pyridine motifs into a picolinamide framework further increases structural complexity and may provide complementary interaction sites capable of engaging enzyme or receptor binding pockets [6]. Consequently, systematic modification of such scaffolds represents a useful approach for identifying new molecular entities with potential cytotoxic and antimicrobial properties. The biological potential of pyridine-containing heterocycles has encouraged the exploration of structurally diverse analogues against cancer and infectious diseases, where optimization of molecular interactions through rational substituent modification remains an important objective in medicinal chemistry [7].

Cancer continues to represent a major therapeutic challenge, and the development of chemically diverse small molecules capable of selectively suppressing cancer-cell proliferation remains an active area of research [8]. Conventional anticancer agents are frequently associated with limitations such as systemic toxicity, resistance, and inadequate selectivity, thereby motivating the search for new structural classes with improved biological profiles. Heterocyclic compounds are particularly attractive in this context because their electronic and structural properties can be readily tuned through substitution. Evaluation against different cancer cell models can provide an initial indication of the ability of newly developed scaffolds to interfere with cellular viability and proliferation [9]. The MTT assay is a widely employed colorimetric approach for preliminary cytotoxicity assessment, in which metabolically active cells reduce tetrazolium salt to formazan, allowing cellular viability to be quantified spectrophotometrically [10]. In the present study, three human cancer cell lines representing breast, colon, and lung cancers—MCF-7, HT-29, and A549, respectively—were selected for evaluation of the synthesized compounds.

In parallel with anticancer drug discovery, the increasing prevalence of bacterial resistance has intensified the need for new antimicrobial chemotypes. Structural diversification of heteroaromatic scaffolds provides an opportunity to identify compounds with activity against bacteria possessing different cellular characteristics [11]. Pyridine-derived compounds containing amide and amino functionalities may offer multiple sites for molecular interactions with bacterial targets or cellular components. Therefore, simultaneous investigation of cytotoxic and antibacterial properties can provide a broader assessment of the biological potential of a newly developed chemical series. In the present work, antibacterial activity was examined against selected Gram-positive and Gram-negative bacterial strains using a cup-plate assay, with ciprofloxacin employed as the reference antibacterial agent [12].

Despite the considerable interest in biologically active pyridine derivatives, systematic investigation of structurally related picolinic acid-derived carboxamides incorporating a pyridinylamino substituent remains comparatively valuable for establishing preliminary structure–activity relationships. Subtle changes in the substituent attached to the amide nitrogen may substantially influence biological performance by altering steric effects, electronic distribution, hydrogen-bonding interactions, and lipophilicity. Such systematic structural variation can therefore provide insight into the molecular features required for cytotoxic and antibacterial activity. Based on this rationale, the present study was designed to develop a series of N,N-disubstituted-4-(pyridin-2-ylamino) pyridine-2-carboxamide derivatives (IVa–IVj) as structurally related picolinic acid-based heterocyclic compounds [13].

The compounds were obtained through a multistep synthetic strategy involving conversion of picolinic acid to 4-chloropyridine-2-carbonyl chloride, subsequent formation of substituted carboxamides, and nucleophilic aromatic substitution with 4-aminopyridine. The synthesized derivatives were characterized using physicochemical and spectroscopic techniques, including melting-point determination, infrared spectroscopy, ^1H NMR spectroscopy, and mass spectrometry. The resulting compounds were subsequently investigated for in vitro cytotoxic activity against MCF-7, HT-29, and A549 cell lines and antibacterial activity against selected Gram-positive and Gram-negative bacterial strains. The biological data were further considered in relation to structural variation across the synthesized series to identify promising derivatives and provide preliminary structure–activity insights that may guide subsequent optimization of this picolinic acid-derived scaffold.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Picolinic acid was used as the starting material for the synthesis of the target derivatives. Thionyl chloride, anhydrous N,N-dimethylformamide (DMF), toluene, tetrahydrofuran (THF), methanol, potassium tert-butoxide, potassium carbonate, ethyl acetate, sodium sulfate, brine, and other analytical-grade solvents and reagents were used as required for the synthetic procedures. Secondary amines were employed for the preparation of the corresponding N,N-disubstituted carboxamide intermediates. 4-Aminopyridine was used for the construction of the pyridinylamino-substituted final derivatives. All solvents and reagents were used as received unless otherwise specified. For biological evaluation, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine, trypsin, ethylenediaminetetraacetic acid (EDTA), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were used for cell culture and cytotoxicity experiments. Dimethyl sulfoxide (DMSO) was used for preparation of test-compound stock solutions. Nutrient

broth and nutrient agar were used for bacterial culture and antibacterial screening. Ciprofloxacin and cisplatin were employed as reference standards for antibacterial and cytotoxicity experiments, respectively.

2.2. General synthetic strategy

The target picolinic acid-derived carboxamides (IVa–IVj) were synthesized through a three-step synthetic sequence. Initially, picolinic acid (I) was converted into 4-chloropyridine-2-carbonyl chloride (II) under chlorinating conditions. The resulting acid chloride was subsequently reacted with appropriate secondary amines to furnish the corresponding 4-chloro-N,N-disubstituted pyridine-2-carboxamides (III). In the final step, the aryl chloride intermediate was subjected to nucleophilic substitution with 4-aminopyridine under basic conditions to afford the desired N,N-disubstituted-4-(pyridin-2-ylamino) pyridine-2-carboxamide derivatives (IVa–IVj) [14, 15]. The overall synthetic route was used to generate a structurally related series in which variation of the N-substituent provided the basis for subsequent biological evaluation (Fig. 2).

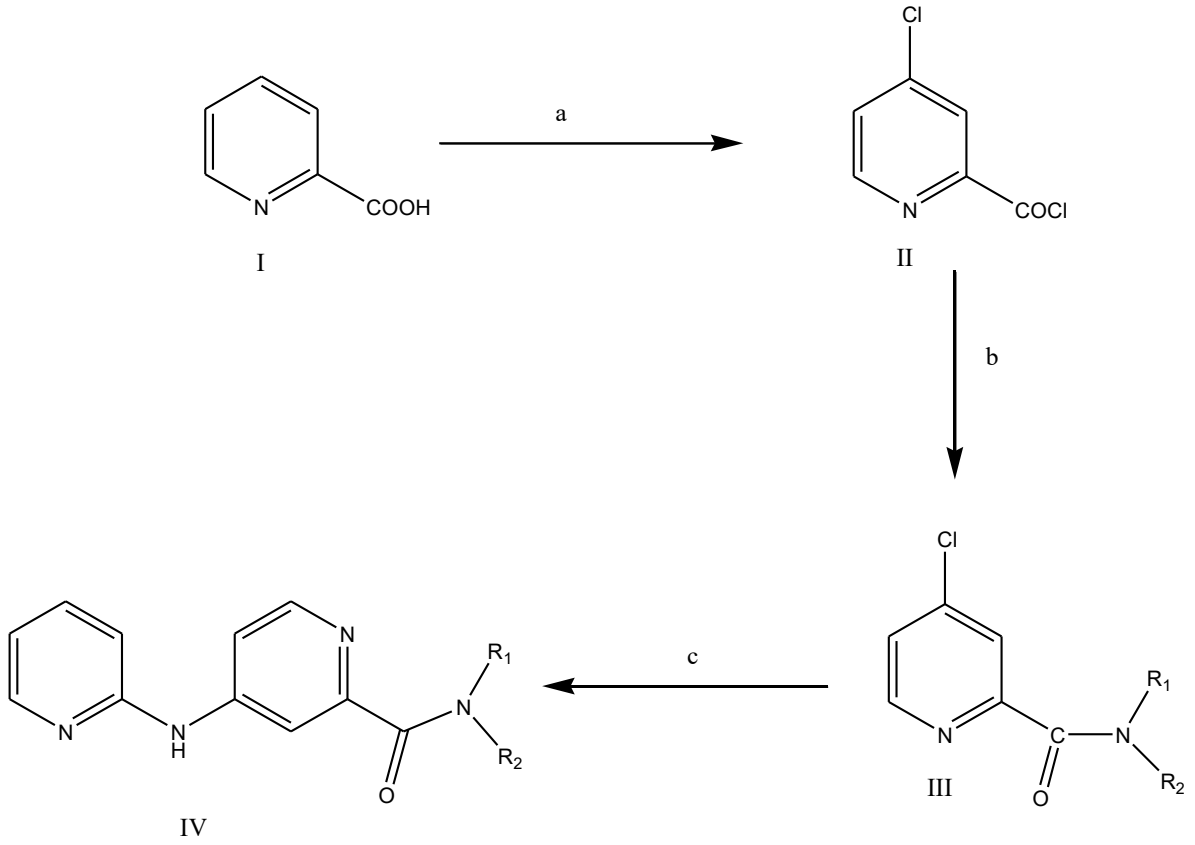


Fig. 2. Synthetic route for the preparation of N,N-disubstituted-4-(pyridin-2-ylamino) pyridine-2-carboxamide derivatives (IV). Reagents and conditions: (a) $SOCl_2$, anhydrous DMF; (b) dimethylamine/appropriate secondary amine, THF, methanol, 0–3 °C, 4 h; (c) t-BuOK, DMF, K_2CO_3, 4-aminopyridine, 80 °C, 6 h. R_1 and R_2 represent the substituents derived from the corresponding secondary amines. The reaction sequence involves conversion of picolinic acid (I) to 4-chloropyridine-2-carbonyl chloride (II), formation of the corresponding 4-chloro-N,N-disubstituted pyridine-2-carboxamide intermediate (III), and subsequent nucleophilic substitution with 4-aminopyridine to furnish the target derivatives (IVa–IVj).

2.2.1. 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 mixture was stirred for 10 min at the same temperature, after which picolinic acid (I) (0.48 mol) was added portionwise over 30 min. During addition, the reaction mixture underwent a progressive color change from green to orange and subsequently purple. The reaction mixture was then heated to 72 °C and maintained under vigorous reaction conditions with evolution of sulfur dioxide. After 16 h, the reaction mixture was cooled to room temperature and diluted with toluene. The solvent was removed by concentration to approximately 200 mL, and the dilution–concentration process was repeated twice. The resulting material was concentrated nearly to dryness, filtered, washed with toluene (50 mL), and dried under high vacuum for 4 h to obtain 4-chloropyridine-2-carbonyl chloride (II). The product was obtained in 80% yield with a melting point of 151–154 °C. The molecular ion was observed at m/z 176.17 (M^+) [16].

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

The synthesized 4-chloropyridine-2-carbonyl chloride (II) (0.03 mol) was treated with a secondary amine solution (2.0 mol/L) in THF and methanol at 0 °C under an argon atmosphere. The reaction mixture was stirred at approximately 3 °C for 4 h and subsequently concentrated to near dryness. The resulting residue was processed to obtain the corresponding 4-chloro-N,N-disubstituted pyridine-2-carboxamide intermediate (III). The intermediate was obtained in 78% yield with a melting point of 166–168 °C. Its molecular ion peak was recorded at m/z 185.21 ($M^+ + 1$) [17].

2.2.3. Synthesis of N,N-disubstituted-4-(pyridin-2-ylamino) pyridine-2-carboxamides (IVa–IVj)

4-Aminopyridine (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) and potassium carbonate were added, and the 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 layers were separated, and the aqueous phase was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated to obtain the corresponding N,N-disubstituted-4-(pyridin-2-ylamino) pyridine-2-carboxamide derivative (IV). Using this general procedure, ten derivatives (IVa–IVj) were prepared. The individual physical characteristics, molecular formulae, melting points, and isolated yields were recorded for each derivative. The isolated yields of the synthesized compounds ranged from 63 to 72%, indicating satisfactory conversion across the series [18].

2.2.4. Physicochemical and spectroscopic characterization

The synthesized compounds were characterized using physicochemical and spectroscopic parameters. Melting points were determined for the synthesized derivatives and are reported as an indicator of their physical characteristics. Infrared (IR) spectroscopy was used to identify characteristic functional-group absorptions, while ^1H nuclear magnetic resonance (^1H NMR) spectroscopy was employed to assess the proton environment and structural features of representative compounds. Mass spectrometry was used to determine the molecular-ion or protonated molecular-ion signals and thereby support the proposed molecular structures. For the representative compound IVa, the IR spectrum recorded using KBr showed characteristic absorptions at 3133.1 cm^{-1} corresponding to aromatic C–H stretching, 1608.5–1512.1 cm^{-1} attributable to C=C/C=N stretching, 1236.3 cm^{-1} associated with C–N stretching, 1661.6 cm^{-1} corresponding to the amide carbonyl group, and 3253.7 cm^{-1} associated with N–H stretching. The ^1H NMR spectrum recorded in DMSO-d_6 showed aromatic proton signals at δ 7.0–7.9 ppm, an N–H signal at δ 4.6 ppm, and a dimethylamino signal at δ 2.7 ppm. The molecular ion was observed at m/z 243.15 ($M^+ + 1$) [19, 20].

2.3. In vitro cytotoxicity evaluation

2.3.1. Cell lines and culture conditions

The cytotoxic potential of compounds IVa–IVj 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. Cells were maintained as adherent monolayers in DMEM supplemented with 10% FBS, 100 µg/mL penicillin, 200 µg/mL streptomycin, and 2 mM L-glutamine. Cultures were maintained in a humidified incubator at 37 °C under 5% CO_2.

2.3.2. Preparation of test solutions

Stock solutions of the synthesized compounds were 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 µg/mL. The resulting solutions were used for treatment of the respective cancer cell lines [21].

2.3.3. MTT assay

Cell viability was determined using the MTT colorimetric assay. Briefly, viable cells were quantified using the trypan blue exclusion method, and approximately 1×10^4 cells were seeded into each well of a 96-well tissue-culture plate. Following cell attachment, the cells were exposed to different concentrations of the test compounds and incubated at 37 °C for the specified treatment period. After treatment, the culture medium was replaced with 90 µL of fresh serum-free medium, followed by addition of 10 µL of MTT reagent (5 mg/mL). The plates were incubated at 37 °C for 4 h to permit formation of insoluble formazan crystals by metabolically active cells. The medium was subsequently removed, and 200 µL of DMSO was added to each well to dissolve the formed formazan crystals. The plates were further incubated at 37 °C for 10 min, and absorbance was measured at 570 nm using a SpectraMax microplate spectrophotometer (Molecular Devices). The percentage inhibition of cell viability was determined relative to the untreated control, and IC_{50} values were calculated from the concentration–response relationship. Cisplatin was used as the reference cytotoxic agent. The reported IC_{50} values for compounds IVa–IVj and cisplatin were used for comparative assessment of cytotoxic potency across the three cancer cell lines [22]

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2.4. In vitro antibacterial activity

The antibacterial activity of the synthesized derivatives was evaluated using the cup-plate method against two Gram-positive bacterial strains, Bacillus thermophilus (ATCC 7953) and Staphylococcus aureus (ATCC 25923), and two Gram-negative strains, Klebsiella pneumoniae (MTCC 3384) and Salmonella paratyphi A (ATCC 11511). Ciprofloxacin was used as the reference antibacterial agent. Nutrient broth was used for preparation of bacterial inocula, while nutrient agar was used for the antibacterial screening assay. 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 the final pH adjusted to 7.4 ± 0.2. The bacterial strains were subcultured on nutrient agar and incubated at 37 ± 1 °C for 24 h. For preparation of inocula, a loopful of each stock culture was transferred into 100 mL of nutrient broth and incubated at 37 ± 1 °C for 48 h [23].

Test solutions were prepared by dissolving 10 mg of each synthesized compound in 10 mL of dimethylformamide, giving a nominal concentration of 1 mg/mL. Ciprofloxacin was prepared separately in sterile distilled water. Nutrient agar medium was sterilized by autoclaving at 121 °C and 15 lb/in² for 15 min. Sterile Petri plates were prepared by aseptically pouring approximately 27 mL of molten nutrient agar into each plate. The bacterial inoculum was incorporated into the medium before solidification. After solidification, sterile cork borers were used to prepare wells in the agar, and 0.1 mL of each test solution was added to the respective well. The plates were maintained under refrigerated conditions for approximately 2 h to facilitate diffusion of the test compounds and subsequently incubated at 37 ± 1 °C for 24 h. The diameter of the resulting zones of inhibition was measured using an antibiotic zone reader. All experiments were performed in triplicate. A solvent control containing 0.1 mL of DMF was included to determine any contribution of the solvent to antibacterial activity [24].

2.5. Data presentation and biological activity assessment

The cytotoxic activity was expressed as IC_{50} values in µM, with lower IC_{50} values indicating greater inhibition of cellular viability. Antibacterial activity was expressed as the diameter of the zone of inhibition in millimetres at a test concentration of 200 µg/cup. The biological activity of derivatives IVa–IVj was compared with the respective reference standards, cisplatin for cytotoxicity and ciprofloxacin for antibacterial activity. The resulting activity profiles were subsequently examined in relation to structural variation among the synthesized picolinic acid-derived carboxamides to provide preliminary structure–activity relationship insights.

2.6. Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical significance between groups was evaluated using one-way analysis of variance (ANOVA), followed by an appropriate post-hoc test where applicable. A p value < 0.05 was considered statistically significant.

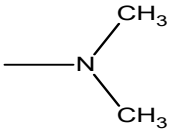
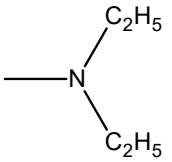
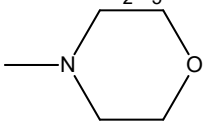
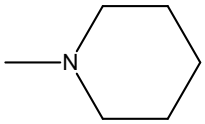
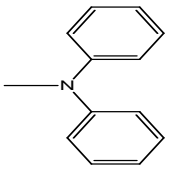
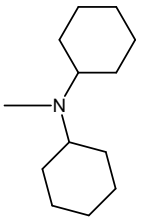
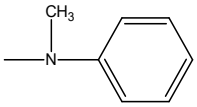
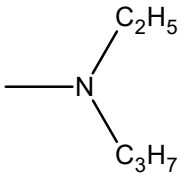
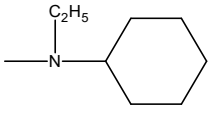
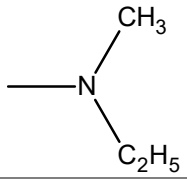
3. RESULTS AND DISCUSSION

3.1. Chemistry and synthesis

A series of ten novel picolinic acid-derived heteroaromatic carboxamide derivatives (IVa–IVj) was prepared through a sequential synthetic strategy involving acid chloride formation, amidation, and nucleophilic aromatic substitution. Picolinic acid (I) was initially converted to 4-chloropyridine-2-carbonyl chloride (II), which was subsequently reacted with appropriate secondary amines to afford the corresponding 4-chloro-N,N-disubstituted pyridine-2-carboxamide intermediates (III). The final derivatives (IVa–IVj) were obtained by reaction of intermediates III with 4-aminopyridine under basic conditions. The overall synthetic sequence provided the target compounds in moderate-to-good isolated yields. The first-stage conversion of picolinic acid to intermediate II afforded the product in 80% yield, with a melting point of 151–154 °C and a molecular-ion signal at m/z 176.17 (M^+). Subsequent amidation afforded intermediate III in 78% yield, with a melting point of 166–168 °C and a reported molecular-ion signal at m/z 185.21 ($M^+ + 1$). The final coupling/substitution reaction furnished the desired pyridinylamino-picolinamide derivatives.

The ten final derivatives showed isolated yields ranging from 63 to 72%, indicating reproducible synthetic conversion across the series. Compound IVa was obtained in 72% yield with a melting point of 181–183 °C, whereas IVb, IVc, IVd, IVe, IVf, IVg, IVh, IVi, and IVj were obtained in yields of 69, 71, 65, 67, 63, 69, 68, 66, and 71%, respectively. Their melting points ranged from 181 to 225 °C. The molecular formulae and physicochemical characteristics of the individual derivatives are summarized in Table 1.

Table 1. Physicochemical characteristics of the synthesized picolinic acid derivatives (IVa–IVj).

S.No.	Comp.	R	Mol. Formula	Mp	Yield%
1	IVa		C13H14N4O	181-183	72
2	IVb		C15H18N4O	194-196	69
3	IVc		C15H16N4O2	211-214	71
4	IVd		C16H18N4O	205-208	65
5	IVe		C23H18N4O	216-219	67
6	IVf		C23H30N4O	223-225	63
7	IVg		C18H16N4O	191-193	69
8	IVh		C15H18N4O	184-186	68
9	IVi		C19H24N4O	205-207	66
10	IVj		C14H16N4O	187-189	71

3.2. Structural characterization

The synthesized derivatives were characterized by physicochemical and spectroscopic techniques. Representative spectral data supported the proposed pyridinylamino-carboxamide framework. For compound IVa, the IR spectrum exhibited an absorption at 1661.6 cm^{-1} attributable to the amide carbonyl (C=O), together with a band at 3253.7 cm^{-1} corresponding to N–H stretching. Signals observed in the 1608.5–1512.1 cm^{-1} region were assigned to aromatic C=C/C=N stretching vibrations, while the absorption at 1236.3 cm^{-1} was associated with C–N stretching. An aromatic C–H stretching band was observed at 3133.1 cm^{-1}. The ^1H NMR spectrum of IVa recorded in DMSO-d_6 showed multiplet signals in the δ 7.0–7.9 ppm region corresponding to aromatic protons. A signal at δ 4.6 ppm was assigned to the NH proton, while the signal at δ 2.7 ppm was attributed to the N,N-dimethyl group. The mass spectrum showed a molecular-ion-related signal at m/z 243.15 ($M^+ + 1$), providing additional support for the proposed molecular structure.

3.3. In vitro cytotoxic activity

The synthesized compounds IVa–IVj were evaluated for their cytotoxic potential against MCF-7, HT-29, and A549 human cancer cell lines using the MTT assay. Cisplatin was used as the reference cytotoxic agent. The activity was expressed as IC_{50} values, with lower values indicating greater cytotoxic potency. The complete cytotoxicity profile is presented in Table 2. The compounds displayed considerable variation in cytotoxic activity across the three cell lines, indicating that structural modification within the picolinic acid-derived scaffold substantially influenced biological response. IVa showed moderate activity against MCF-7 and HT-29 cells, with IC_{50} values of 90.5 and 89.2 µM, respectively, whereas no activity was reported against A549 cells. IVb exhibited IC_{50} values of 88.8, 94.6, and 81.7 µM against MCF-7, HT-29, and A549 cells, respectively.

A pronounced enhancement in cytotoxic activity was observed for IVc. This derivative exhibited IC_{50} values of 9.2 µM against MCF-7, 7.50 µM against HT-29, and 16.2 µM against A549 cells. Thus, IVc represented the most potent member of the series against both MCF-7 and HT-29 cells and showed substantially improved activity compared with several other derivatives. IVd displayed intermediate cytotoxic activity, with IC_{50} values of 25.6, 28.3, and 35.5 µM against MCF-7, HT-29, and A549 cells, respectively. IVe was comparatively less potent, exhibiting IC_{50} values of 70.4, 68.7, and 59.6 µM against the respective cell lines. IVf showed improved activity relative to IVe, with IC_{50} values of 16.3, 15.9, and 23.8 µM against MCF-7, HT-29, and A549, respectively. IVg showed moderate activity against MCF-7, with an IC_{50} of 70.5 µM, while greater activity was observed against HT-29 and A549, with IC_{50} values of 35.6 and 38.4 µM, respectively. In contrast, IVh demonstrated strong and consistent activity across all three cell lines, with IC_{50} values of 12.8, 9.4, and 17.5 µM against MCF-7, HT-29, and A549, respectively.

IVi showed moderate cytotoxic activity, with IC_{50} values of 54.6, 71.9, and 62.6 µM against MCF-7, HT-29, and A549, respectively. IVj exhibited IC_{50} values of 80.6, 50.4, and 49.6 µM against MCF-7, HT-29, and A549, respectively. Cisplatin produced IC_{50} values of 8.61, 6.56, and 14.31 µM against MCF-7, HT-29, and A549, respectively. Accordingly, IVc and IVh approached the activity of cisplatin, particularly against HT-29 cells, although the reference drug remained more potent overall. Among the tested cancer models, the HT-29 cell line appeared particularly responsive to the most active derivatives. IVc and IVh exhibited IC_{50} values of 7.50 and 9.4 µM, respectively, compared with 6.56 µM for cisplatin. These findings identify IVc and IVh as the principal cytotoxic candidates within the synthesized series and warrant further mechanistic investigation.

Table 2. In vitro cytotoxic activity of synthesized compounds (IVa–IVj) against human cancer cell lines.

	IC50 (µm)		
Compound	**MCF-7**	**HT-29**	**A 549**
IVa	90.5	89.2	NA
IVb	88.8	94.6	81.7
IVc	9.2	7.50	16.2
IVd	25.6	28.3	35.5
IVe	70.4	68.7	59.6
IVf	16.3	15.9	23.8
IVg	70.5	35.6	38.4
IVh	12.8	9.4	17.5
IVi	54.6	71.9	62.6
IVj	80.6	50.4	49.6
Cisplatin	8.61	6.56	14.31

3.3.1. Structure–activity relationship analysis of cytotoxicity

Comparison of the cytotoxicity data across IVa–IVj revealed a clear dependence of biological activity on the structural variation introduced at the carboxamide nitrogen. The activity was not uniformly proportional to the size or hydrophobicity of the substituent, suggesting that an appropriate balance of steric and electronic

properties is important for interaction with cellular targets. IVc emerged as the most potent derivative, displaying sub-20 µM activity against all three cancer cell lines. IVh showed a highly comparable profile, whereas IVf also demonstrated relatively strong activity. In contrast, IVa, IVb, IVe, IVg, IVi, and IVj generally exhibited weaker activity, with several IC_{50} values exceeding 50 µM. These observations suggest that specific substituent patterns can favorably modulate the biological properties of the pyridinylamino-picolinamide scaffold. However, because the present dataset does not provide mechanistic or molecular-target information, these SAR observations should be regarded as preliminary and hypothesis-generating rather than definitive.

3.4. In vitro antibacterial activity

The antibacterial activity of compounds IVa–IVj was assessed against two Gram-positive strains, Bacillus thermophilus and Staphylococcus aureus, and two Gram-negative strains, Klebsiella pneumoniae and Salmonella paratyphi A. Activity was expressed as the diameter of the zone of inhibition at 200 µg/cup. Ciprofloxacin (10 µg/cup) was used as the reference standard. The antibacterial results are summarized in Table 3. IVa and IVb exhibited relatively weak antibacterial activity, with inhibition zones ranging from 2 to 6 mm across the tested organisms. IVd–IVg and IVi–IVj also showed variable and generally moderate-to-low activity, with some compounds exhibiting no detectable activity against particular bacterial strains. For example, IVd showed no activity against S. aureus, while IVe showed no activity against S. paratyphi A. IVg showed no detectable activity against K. pneumoniae.

In contrast, IVc exhibited the strongest antibacterial profile among the synthesized derivatives. It produced inhibition zones of 14, 15, 12, and 15 mm against B. thermophilus, S. aureus, K. pneumoniae, and S. paratyphi A, respectively. This broad-spectrum activity across both Gram-positive and Gram-negative organisms distinguishes IVc from most of the other synthesized derivatives. IVh also demonstrated pronounced antibacterial activity, producing inhibition zones of 12, 14, 11, and 10 mm against B. thermophilus, S. aureus, K. pneumoniae, and S. paratyphi A, respectively. Thus, IVh represented the second most active derivative across the tested bacterial panel. The reference compound 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 IVc and IVh demonstrated appreciable antibacterial activity, their inhibition zones remained lower than those produced by ciprofloxacin under the reported assay conditions.

Table 3. In vitro antibacterial activity of synthesized compounds (IVa–IVj)

Compound	Gram +ve		Gram –ve	
	Bacillus thermophilus	***Staphylococcus aureus***	***Klebsiella pneumoniae***	***Salmonella paratyphi A***
IVa	4	3	5	3
IVb	5	3	6	2
IVc	14	15	12	15
IVd	3	NA	5	2
IVe	5	8	4	NA
IVf	3	6	3	5
IVg	5	3	-	3
IVh	12	14	11	10
IVi	4	7	7	3
IVj	6	3	7	5
Ciprofloxacin 10µg/cup	18	20	16	18

3.5. Comparative biological profile

An integrated comparison of the cytotoxic and antibacterial data identified IVc and IVh as the most promising derivatives of the series. IVc combined strong cytotoxic activity against MCF-7, HT-29, and A549 cells with the highest antibacterial activity observed among the synthesized compounds. Its IC_{50} values were 9.2, 7.50, and 16.2 µM against MCF-7, HT-29, and A549, respectively, while its antibacterial inhibition zones ranged from 12 to 15 mm. IVh displayed a similarly favorable dual biological profile, with IC_{50} values of 12.8, 9.4, and 17.5 µM and antibacterial inhibition zones of 10–14 mm across the respective test organisms. The concordance between cytotoxic and antibacterial activity suggests that the structural features present in IVc and IVh may be particularly favorable for biological interactions. Nevertheless, the present findings represent preliminary in vitro evidence, and further studies involving mechanistic assays, selectivity evaluation against nonmalignant cells, minimum inhibitory concentration determination, and molecular-level investigations would be required to establish their therapeutic potential. Overall, the biological screening demonstrates that structural diversification of the picolinic acid-derived pyridinylamino-carboxamide scaffold can produce substantial differences in biological activity. Among the synthesized derivatives, IVc and IVh emerged as the most promising compounds, providing a basis for further structural optimization and mechanistic investigation of this heteroaromatic scaffold.

4. CONCLUSION

A series of ten N,N-disubstituted-4-(pyridin-2-ylamino) pyridine-2-carboxamide derivatives (IVa–IVj) was successfully prepared through a concise multistep synthetic route from picolinic acid and obtained in moderate-to-good yields. Spectroscopic and physicochemical characterization supported the proposed structures. Biological evaluation demonstrated that structural modification of the carboxamide substituent markedly influenced both cytotoxic and antibacterial activities. Among the synthesized derivatives, IVc and IVh emerged as the most promising compounds, exhibiting pronounced cytotoxic activity against MCF-7, HT-29, and A549 cells and appreciable antibacterial activity against the tested Gram-positive and Gram-negative strains. IVc showed the highest overall biological activity, particularly against HT-29 cells and across the bacterial panel. Although their activities remained lower than those of the respective reference standards, these findings demonstrate the biological potential of the picolinic acid-derived pyridinylamino-carboxamide scaffold. Further optimization, mechanistic studies, selectivity assessment against non-cancerous cells, and comprehensive antimicrobial evaluation are warranted to establish the therapeutic relevance of these compounds.

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