Indian Journal of Chemistry
Vol. 65, August 2026, 783-790
DOI: 10.56042/ijc.v65i8.26599

Exploring imidazo[1,2-a]pyridine–thiazole pyrazolines as potent anticancer and antimicrobial agents

Samadhan A Shenmare[a,b], Dnyaneshwar M Sirsat[c], Nikita N Mali[d], Sadanand N Shringare[a], Tukaram D Jadhav[d], Raghunath B Bhosale[a] & Pravin S Bhale*[b]

[a] Organic Chemistry Research Laboratory, School of Chemical Sciences, Punyashlok Ahilyadevi Holkar Solapur University, Solapur 413 255, Maharashtra, India

[b] Department of Chemistry, Yeshwantrao Chavan Mahavidyalaya, Tuljapur, Dist. Dharashiv 413 601, Maharashtra, India

[c] Department of Chemistry, Anandibai Raorane Arts, Commerce and Science College, Vaibhavwadi, Dist. Sindhudurg 416 810, Maharashtra, India

[d] Department of Chemistry, Sub-Campus, Dr. Babasaheb Ambedkar Marathwada University, Dharashiv 413 501, Maharashtra, India

E-mail: bhale.ps@gmail.com

Received 15 December 2025; accepted (revised) 14 August 2026

A novel series of imidazo[1,2-a]pyridine–thiazole hybrid pyrazolines **4a–l** has been synthesized *via* Claisen–Schmidt condensation of thiazolyl ketones with imidazo[1,2-a]pyridine aldehydes, followed by cyclization with hydrazine hydrate. Structures have been confirmed by FT-IR, ^{1}H and ^{13}C NMR, and HRMS. The compounds have been evaluated for *in vitro* anticancer activity against MCF-7 cells using the SRB assay. Most derivatives show limited cytotoxicity (GI_{50} >100 µM), while cyano-substituted analogues exhibit improved potency. Compound **4k**, featuring a *para*-chloro group on the thiazole phenyl ring and a cyano group on the imidazo[1,2-a]pyridine moiety, displays the highest activity (GI_{50} = 21.0 µM), highlighting the impact of electron-withdrawing substituents on activity. Antimicrobial screening *via* the disc diffusion method reveals moderate to significant antibacterial activity, particularly against Gram-positive strains, with compound **4l** showing the largest inhibition zones (32 mm). Activity against Gram-negative bacteria and *Candida albicans* is moderate. These findings demonstrate that imidazo[1,2-a]pyridine–thiazole hybrid pyrazolines possess dual anticancer and antimicrobial potential, offering promising scaffolds for further medicinal chemistry optimization.

Keywords: Imidazo[1,2-a]pyridine, Thiazole, Pyrazoline, Anti-cancer activity, Antimicrobial activity

Cancer remains a major global health challenge and a leading cause of mortality worldwide. The World Health Organization (WHO) reported nearly 10 million cancer-related deaths in 2020, with approximately 20 million new cases and 9.7 million deaths in 2022, and projections indicate a 77% rise in incidence by 2050 (Ref. 1,2). Among malignancies, lung, breast, and colorectal cancers were the most commonly diagnosed, while lung cancer accounted for the highest mortality, followed by colorectal, liver, and breast cancers[2]. These statistics underscore the increasing global burden of cancer and the urgent need for more effective therapeutic strategies. Breast cancer, a heterogeneous malignancy of mammary epithelial cells, remains the most frequently diagnosed carcinoma and a leading cause of cancer-related deaths among women despite advances in early detection and treatment[3,4]. To address the limitations of conventional therapies, molecular hybridization has emerged as a promising approach in drug design, combining two or more pharmacologically active moieties within a single molecule to simultaneously modulate multiple targets[5]. This strategy offers potential advantages such as enhanced potency, selectivity, and reduced off-target effects, making it a valuable tool for developing novel anticancer agents with improved therapeutic efficacy.

Heterocyclic compounds containing nitrogen and sulfur atoms hold a prominent position in modern medicinal chemistry due to their structural diversity and extensive biological relevance. Among these, imidazo[1,2-a]pyridine, thiazole, and pyrazoline scaffolds have gained particular attention because they exhibit a broad range of pharmacological activities, including anticancer, antimicrobial, anti-inflammatory, antioxidant, and enzyme inhibitory properties. Their favorable physicochemical characteristics, tunable electronic properties, and strong binding affinity toward various biological targets make them ideal pharmacophores for the development of clinically valuable drug candidates.

Imidazo[1,2-a]pyridine derivatives have gained considerable interest due to their fused heterocyclic framework and broad spectrum of biological activities[6]. This scaffold exhibits strong adaptability for pharmacophore modification and interacts effectively with diverse biological targets, contributing to its wide-ranging pharmacological actions, including antimicrobial, anti-inflammatory, antiviral, antiepileptic, antitumor, and anticancer effects[7,8]. Thiazole-containing compounds exhibit diverse biological activities—including anti-inflammatory, anticancer, antimicrobial, antifungal, and antioxidant effects—owing to their distinctive electronic properties and dual heteroatom composition, which facilitate strong interactions with biological targets[9–11]. Pyrazolines, the partially saturated analogues of pyrazoles, further enhance drug-like potential through increased basicity, conformational flexibility, and broad enzyme–receptor affinity, as reflected in several FDA-approved drugs such as edaravone, celecoxib, and phenylbutazone[12–15].

In our earlier investigations, we reported several biologically active molecular hybrids exhibiting noteworthy anticancer properties[16–19]. Building on these results and in continuation of our ongoing efforts to develop novel bioactive anticancer agents[20–26], we have now synthesized a new series of imidazo[1,2-a]pyridine–thiazole-based pyrazoline hybrids and evaluated their *in vitro* anticancer and antimicrobial activity.

Experimental Section

Materials and Methods

All reagents and solvents used in the synthesis were of synthetic grade and obtained from commercial suppliers. Reaction progress was monitored by thin-layer chromatography (TLC) using silica gel 60 F_{254} aluminum-backed plates (Merck), with spot visualization performed under UV light and/or iodine vapors. All solvents were dried prior to use with appropriate drying agents.Melting points were determined using the open-end capillary method and are reported without correction. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker AV-400 MHz spectrometer, and ^{13}C NMR spectra were obtained at 75 MHz. Chemical shifts (δ, ppm) were referenced to tetramethylsilane (TMS) as an internal standard. IR spectra were recorded on a Shimadzu FT-IR spectrophotometer using 1% (w/w) potassium bromide (KBr) pellets. Mass spectra were acquired on a Shimadzu LCMS-2010EV instrument (Shimadzu, Japan). *In vitro* anticancer activity assays were conducted at the Tata Memorial Centre, Advanced Centre for Treatment, Research, and Education in Cancer (ACTREC), Kharghar, Navi Mumbai 410 210.

Synthesis

General procedure for the synthesis of imidazo[1,2-a]pyridine-thiazole hybrid pyrazolines, 4a-l

Imidazo[1,2-a]pyridine-based thiazole chalcones (**3a-l**) (1.0 mmol) were dissolved in ethanol (15 mL), followed by the addition of hydrazine hydrate (2.0 mmol). The reaction mixture was refluxed at 80-90°C for 7-8 h under continuous magnetic stirring, and the progress was monitored by thin-layer chromatography (TLC). Upon completion, the reaction mixture was cooled to RT and then poured into ice-cold water, followed by neutralization with dilute acetic acid. The precipitated solid was collected by filtration, washed with cold water, and dried. The crude product was purified by recrystallization from ethanol to afford the corresponding hybrid pyrazolines (**4a-l**).

Spectral data of representative compounds

2-(4-Fluoro-phenyl)-3-[5-(4-methyl-2-phenyl-thiazol-5-yl)-3,4-dihydro-2*H*-pyrazol-3-yl]-imidazo[1,2-a]pyridine, 4a: Pale Yellow solid. Yield 90%. 326-328°C. IR (KBr): 3278 (N-H), 1632 (C=N), 1559 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.69 (s, 1H), 8.17 (s, 1H), 7.97-7.95 (m,2H), 7.87-7.81 (m, 2H), 7.53-7.43 (m, 4H), 7.22-7.00 (m, 3H), 6.16 (d, *J* = 9.2Hz,1H), 5.73 (m, *J* = 6.8Hz, 1H), 3.88-3.40 (m, 2H, -CH_2), 2.70 (s, 3H, -CH_3); HRMS: Calcd for $C_{26}H_{20}FN_5S$; Exact mass: 453.1423. Found (ESI M+H); HRMS: *m/z* 454.1457 (M+H).

3-{5-[2-(4-Chloro-phenyl)-4-methyl-thiazol-5-yl]-3,4-dihydro-2*H*-pyrazol-3-yl}-2-(4-fluoro-phenyl)-imidazo[1,2-a]pyridine, 4b: Pale Yellow solid. Yield 88%. 320-322°C. IR (KBr): 3270 (N-H), 1499 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.59 (d, *J* = 6.4Hz, 1H), 8.14 (d, *J* = 8.4Hz, 1H), 7.94-7.83 (m, 3H), 7.77-7.67 (m, 1H), 7.46-7.40 (m, 3H), 7.32-7.12(m, 4H,),5.76-5.69 (m, 1H), 3.58-3.41 (m, 2H, -CH_2), 2.67(s, 3H, -CH_3); HRMS: Calcd for $C_{26}H_{19}ClFN_5S$; Exact mass: 487.1034. Found (ESI M+H); HRMS: *m/z* 488.1087 (M+H).

4-(2-(4-Fluorophenyl)-*H*-imidazo[1,2-a]pyridin-3-yl)-6-(2-(4-methoxyphenyl)-4-methylthiazol-5-yl)pyrimidin-2-amine, 4c: Pale Yellow solid. Yield 80%. 330-332°C. IR (KBr): 3263 (N-H), 1607 (C=N), 1505 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ

8.15 (d, *J* = 8.0 Hz, 1H), 7.92-7.88 (m,2H), 7.79 (d, *J* = 7.6 Hz, 2H), 7.22-7.15 (m, 4H), 7.00-6.95 (m, 3H), 6.14 (s, 1H), 5.72 (s, 1H), 3.88 (s, 3H, -OCH_3), 3.61-3.48 (m,2H, -CH_2), 2.67 (s, 3H, -CH_3); HRMS: Calcd for $C_{27}H_{22}FN_5OS$; Exact mass: 483.1529. Found (ESI M+H); HRMS: *m/z* 484.1329 (M+H).

3-[5-(4-Methyl-2-phenyl-thiazol-5-yl)-3,4-dihydro-2*H*-pyrazol-3-yl]-2-*p*-tolyl-imidazo[1,2-a]pyridine, 4d: Pale Yellow solid. Yield 87%. 332-334°C. IR (KBr): 3265 (N-H), 1497 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.43 (d, *J* = 7.2 Hz, 1H), 7.95 (d, *J* = 7.6Hz, 1H), 7.88 (d, *J* = 8.4Hz, 1H), 7.78 (d, *J* = 8.4Hz, 1H), 7.55 (d, *J* = 6.4Hz, 2H), 7.46-7.41 (m, 3H), 7.31-7.27 (m, 3H), 6.88 (d, *J* = 6.4Hz, 1H),5.97 (s, 1H), 5.8.-5.72 (m, 1H), 3.49-3.46 (m, 2H, -CH_2), 2.67 (s, 3H, -CH_3), 2.42 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.58, 151.94, 146.69, 146.46, 138.11, 136.26, 131.74, 130.32, 129.41, 129.24, 129.00, 128.79, 127.65, 126.49, 126.19, 125.73, 124.81, 117.85, 115.85, 56.64, 38.29, 21.31, 17.34; HRMS: Calcd for $C_{27}H_{23}N_5S$. Exact mass: 449.1674. Found (ESI M+H); HRMS: *m/z* 475.1710 (M+H).

4-(2-(4-Chlorophenyl)-4-methylthiazol-5-yl)-6-(2-*p*-tolyl-*H*-imidazo[1,2-a]pyridin-3-yl)pyrimidin-2-amine, 4e: Pale Yellow solid. Yield 89%. 326-328°C. IR (KBr): 3271 (N-H), 1631 (C=N), 1562 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.48 (d, *J* = 6.8 Hz, 1H), 7.93-7.87 (m,3H), 7.59-7.53 (m, 2H), 7.45-7.41 (m, 3H), 7.31 (d, *J* = 7.6 Hz, 2H), 7.00-6.96 (m, 1H), 6.06 (s, 1H, -NH), 5.80-5.72 (m, 1H), 3.50-3.47 (m,2H, -CH_2), 2.67 (s, 3H, -CH_3), 2.42 (s, 3H,-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.39, 164.62, 162.23, 151.94, 146.45, 138.07, 136.26, 131.73, 129.62, 129.41, 129.23, 128.79, 127.64, 126.14, 125.72, 124.89, 117.80, 112.22, 110.20, 56.61, 38.29, 21.30, 17.54; HRMS: Calcd for $C_{27}H_{22}ClN_5S$; Exact mass: 483.1284. Found (ESI M+H); HRMS: *m/z* 484.1357 (M+H).

3-{5-[2-(4-Methoxy-phenyl)-4-methyl-thiazol-5-yl]-3,4-dihydro-2*H*-pyrazol-3-yl}-2-*p*-tolyl-imidazo[1,2-a]pyridine, 4f: Pale Yellow solid. Yield 92%. 336-328°C. IR (KBr): 3258 (N-H), 1604 (C=N), 1506 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.49 (d, *J* = 6.8 Hz, 1H), 7.91-7.87 (m,3H), 7.58 (d, *J* = 8.0Hz, 2H), 7.32-7.27 (m,3H), 6.98-6.95 (m, 3H), 5.96 (s, 1H, -NH), 5.79-5.72 (m, 1H), 3.87 (s, 3H, -OCH_3), 3.51-3.48 (m,2H, -CH_2), 2.66 (s, 3H, -CH_3), 2.42 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.11, 160.40, 155.01, 151.41, 149.70, 146.83, 138.14, 129.42, 128.80, 128.04, 126.24, 126.18, 124.32, 117.74, 116.04, 114.34, 112.23, 111.30, 110.04, 56.51, 55.44, 38.40, 21.31, 17.56; HRMS: Calcd for $C_{28}H_{25}N_5OS$; Exact mass: 479.1780. Found (ESI M+H); HRMS: *m/z* 480.1813 (M+H).

2-(4-Chloro-phenyl)-6-methyl-3-[5-(4-methyl-2-phenyl-thiazol-5-yl)-3,4-dihydro-2*H*-pyrazol-3-yl]-imidazo[1,2-a]pyridine, 4g: Pale Yellow solid. Yield 85%. 318-320°C. IR (KBr): 3365 (N-H), 1642 (C=N), 1493 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.22 (s, 1H), 7.97-7.94 (m, 2H), 7.70-7.61 (m, 3H), 7.49-7.43 (m, 5H), 7.20 (d, *J* = 9.2Hz, 1H), 6.11 (s, 1H, -NH), 5.72-5.65 (m, 1H), 3.47-3.44 (m, 2H, -CH_2), 2.69 (s, 3H, -CH_3), 2.36 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.32, 166.26, 151.95, 146.76, 144.89, 134.14, 133.22, 130.36, 130.10, 129.15, 129.01, 128.86, 128.38, 126.50, 125.24, 123.80, 122.19, 117.17, 116.00, 56.61, 38.37, 18.60, 17.60; HRMS: Calcd for $C_{27}H_{22}ClN_5S$: Exact Mass: 483.1284. Found (ESI M+H); HRMS: *m/z* 484.1357 (M+H).

2-(4-Chloro-phenyl)-3-{5-[2-(4-chloro-phenyl)-4-methyl-thiazol-5-yl]-3,4-dihydro-2*H*-pyrazol-3-yl}-6-methyl-imidazo[1,2-a]pyridine, 4h: Pale Yellow solid. Yield 80%. 338-340°C. IR (KBr): 3259 (N-H), 1491 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.37 (s, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 7.92-7.87 (m, 2H), 7.77 (s, 1H), 7.70-7.67 (m, 1H), 7.47-7.39 (m, 5H), 6.13-6.08 (m,1H), 5.75-5.72 (m, 1H), 3.49-3.34 (m, 2H, -CH_2), 2.68 (s, 3H, -CH_3), 2.46 (s, 3H, -CH_3); HRMS: Calcd for $C_{27}H_{21}Cl_2N_5S$: Exact Mass: 517.0895. Found (ESI M+H); HRMS: *m/z* 518.0966 (M+H).

2-(4-Chloro-phenyl)-3-{5-[2-(4-methoxy-phenyl)-4-methyl-thiazol-5-yl]-3,4-dihydro-2*H*-pyrazol-3-yl}-6-methyl-imidazo[1,2-a]pyridine, 4i: Pale Yellow solid. Yield 90%. 334-336°C. IR (KBr): 3415 (N-H), 1605 (C=N), 1512 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.38 (s, 1H), 7.10-7.08 (m, 1H), 7.98-7.77 (m, 2H), 7.75-7.66 (m, 2H), 7.53-7.32 (m, 3H), 7.00-6.95 (m, 2H), 6.16-6.12 (m, 1H), 5.70-5.68 (m, 1H), 3.88 (s, 3H, -OCH_3), 3.54-3.35 (m, 2H, -CH_2), 2.67 (s, 3H, -CH_3), 2.45 (s, 3H, -CH_3); HRMS: Calcd for $C_{28}H_{24}ClN_5OS$: Exact Mass: 513.1390, found (ESI M+H); HRMS: *m/z*514.1424 (M+H).

4-{6-Methyl-3-[5-(4-methyl-2-phenyl-thiazol-5-yl)-3,4-dihydro-2*H*-pyrazol-3-yl]-imidazo[1,2-a]pyridin-2-yl}-benzonitrile, 4j: Pale Yellow solid. Yield 84%. 278-280°C. IR (KBr): 3331 (N-H), 2223

(CN), 1605 (C=N), 1552 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.04-8.02 (m, 1H), 7.98-7.95 (m, 2H), 7.93-7.92 (m, 3H), 7.89-7.86 (m, 4H), 7.78-7.62 (m, 2H), 6.15-6.11 (m, 1H), 5.76-5.70 (m, 1H), 3.37-3.33(m, 2H, -CH_2), 2.68 (s, 3H, -CH_3), 2.50 (s, 3H, -CH_3); HRMS: Calcd for $C_{28}H_{22}N_6S$. Exact Mass: 474.1627. Found (ESI M+H); HRMS: *m/z* 475.1698 (M+H).

4-(3-{5-[2-(4-Chloro-phenyl)-4-methyl-thiazol-5-yl]-3,4-dihydro-2*H*-pyrazol-3-yl}-6-methyl-imidazo[1,2-a]pyridin-2-yl)-benzonitrile, 4k: Pale Yellow solid. Yield 88%. 286-288°C. IR (KBr): 3274 (N-H), 2224 (CN), 1605 (C=N), 1497 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.27 (s, 1H), 7.92-7.87 (m, 5H), 7.77-7.73 (m, 3H), 7.43-7.42 (m, 3H), 5.71-5.70 (m, 1H), 3.48-3.43(m, 2H, -CH_2), 2.67 (s, 3H, -CH_3), 2.41 (s, 3H, -CH_3); HRMS: Calcd for $C_{28}H_{21}ClN_6S$: Exact Mass: 508.1237. Found (ESI M+H); HRMS: *m/z* 509.1304(M+H).

4-(3-{5-[2-(4-Methoxy-phenyl)-4-methyl-thiazol-5-yl]-3,4-dihydro-2*H*-pyrazol-3-yl}-6-methyl-imidazo[1,2-a]pyridin-2-yl)-benzonitrile, 4l: Pale Yellow solid. Yield 87%. 248-250°C. IR (KBr): 3326 (N-H), 2224 (CN), 1666 (C=N), 1512 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.22 (s, 1H), 7.99-7.82 (m, 5H), 7.71-7.60 (m, 3H), 7.52-7.46 (m, 3H), 5.64-5.59(m, 1H), 3.71 (s, 3H, -OCH_3), 3.41-3.35(m, 2H, -CH_2), 2.72(s, 3H, -CH_3), 2.36(s, 3H, -CH_3); HRMS: Calcd for $C_{29}H_{24}N_6OS$. Exact Mass: 504.1732. Found (ESI M+H); HRMS: *m/z* 505.1809 (M+H).

Procedure of the SRB-assay for anticancer screening

The human breast cancer cell line MCF-7 (Source: NCI, USA; NCCS, Pune) was cultured in tissue culture flasks using RPMI-1640 medium supplemented with 2 mM glutamine (*p*H 7.4), 10% fetal calf serum, 100 µg/mL streptomycin, and 100 units/mL penicillin. Cells were maintained at 37°C in a CO_2 incubator under an atmosphere of 5% CO_2 and 95% relative humidity. At the sub-confluent stage, cells were harvested using 0.05% trypsin in PBS containing 0.02% EDTA and resuspended in fresh medium. Only cultures showing >97% viability, as assessed by the trypan blue exclusion method, were used for cytotoxicity studies. An aliquot of 100 µL, containing approximately 5×10^3 cells/well, was seeded into a 96-well plate and allowed to adhere for 24 h. After cell attachment, test compounds at various concentrations were added, and incubation was continued for an additional 48 h under the same environmental conditions. Appropriate blanks and positive controls were included, and all experiments were performed in triplicate. Following incubation, cell growth was terminated by carefully adding 50 µL of 50% trichloroacetic acid (TCA) to each well. The plates were incubated at 4°C for 1 h to allow complete cell fixation. The supernatant was aspirated, and the wells were washed five times with double-distilled water to remove residual TCA and culture medium. The plates were then air-dried. Each well was stained with 100 µL of 0.4% sulforhodamine B (SRB) prepared in 1% acetic acid, followed by incubation at RT for 30 min. Excess dye was removed by washing the plates five times with 1% acetic acid, and the plates were again air-dried. The protein-bound dye was solubilized by adding 100 µL of 0.01 M Tris buffer (*p*H 10.4) to each well, and the plates were gently shaken for 5 min. Absorbance was measured at 540 nm using a microplate (ELISA) reader. Cell growth in untreated control wells was considered 100%, and percentage growth inhibition for each compound was calculated accordingly. GI_{50} values (concentration producing 50% growth inhibition) were determined by regression analysis.

Disc diffusion method

Inocula of each bacterial and fungal pathogen were prepared by overnight incubation in nutrient broth at 37°C. The resulting cultures were subsequently used for antimicrobial evaluation. Bacterial suspensions were standardized to the turbidity of a 0.5 McFarland standard using sterile saline. Nutrient agar plates were inoculated by dispensing 200 µL of the adjusted microbial suspension onto the surface and uniformly spreading it with sterile cotton swabs. Antibiotic discs were then aseptically placed onto the inoculated agar using sterile forceps. All synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain a working concentration of 2 mg/mL. A volume of 100 µL of each compound solution was impregnated onto sterile discs. A DMSO-only disc (100 µL) served as the solvent control to verify that the vehicle exhibited no intrinsic antimicrobial activity. The plates were incubated at 37°C for 24 h. Fluconazole and ciprofloxacin were used as standard reference drugs for antifungal and antibacterial assays, respectively. After incubation, antimicrobial activity was determined by measuring the diameter of the inhibition zones surrounding each disc, reflecting the extent of microbial growth suppression.

Results and Discussion

Chemistry

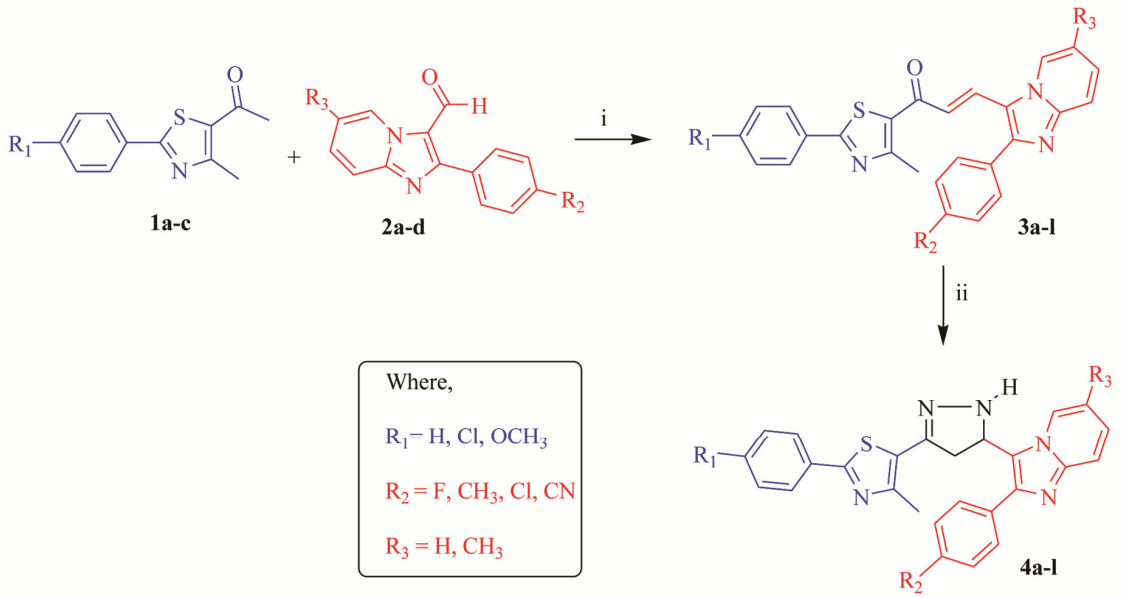
In the present study, a novel series of imidazo[1,2-a]pyridine-integrated thiazole chalcones **(3a-l)**were synthesized through Claisen–Schmidt condensation of substituted thiazolyl ketones **(1a-c)** with substituted imidazo[1,2-a]pyridine aldehydes **(2a-d)** in ethanol using 10% aqueous sodium hydroxide as the base. Subsequent cyclization of these chalcones **(3a-l)** with hydrazine hydrate in refluxing ethanol afforded the corresponding imidazo[1,2-a]pyridine–thiazole hybrid pyrazolines **(4a-l)**, as illustrated in Scheme 1. The thiazolyl ketone precursors **(1a-c)** were obtained in good yields by refluxing substituted thiobenzamides with 3-chloro-2,4-pentanedione in the presence of sodium hydroxide, following a literature-reported procedure with slight modifications[27]. Crude products were purified by column chromatography on silica gel (100–200 mesh) using 10% ethyl acetate in hexane as the mobile phase. Similarly, substituted imidazo[1,2-a]pyridine aldehydes **(2a-d)** were synthesized in good yields *via* a Vilsmeier–Haack formylation of substituted imidazo[1,2-a]pyridines employing phosphorus oxychloride and dimethylformamide as the reaction system[28]. The structures of all synthesized compounds were unequivocally established using FT-IR, ^{1}H and ^{13}C NMR, and HRMS spectroscopic techniques.

Biological Evaluation

In vitro anticancer activity

The synthesized imidazo[1,2-a]pyridine-thiazole hybrid pyrazolines **(4a-l)** were screened for *in vitro* anticancer activity against the human breast cancer cell line MCF-7 using the sulforhodamine B (SRB) assay[29]. MCF-7 cells were selected as they represent a well-characterized estrogen-receptor–positive breast cancer model and are widely employed for evaluating chalcone and imidazo[1,2-a]pyridine-derived cytotoxic agents. Additionally, previous studies[30] report preferential activity of these scaffolds toward MCF-7, supporting their relevance for initial biological assessment. Three parameters GI_{50}, TGI, and LC_{50}were determined, and the results are summarized in Table 1. GI_{50} corresponds to the concentration required to inhibit cell growth by 50%, TGI indicates complete growth arrest, and LC_{50}denotes the concentration causing 50% cell death. Based on GI_{50} values, compounds were classified as inactive (>100 µM), moderately active (10-100 µM), or highly active (<10 µM).

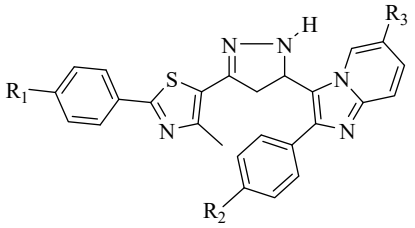
Most synthesized compounds exhibited GI_{50} values above 100 µM, indicating limited cytotoxicity; however, the cyano-substituted derivatives (R_2 = -CN) showed promising activity. Within this subgroup, compound **4j** displayed moderate cytotoxicity (GI_{50} = 54.0 µM), while **4k** emerged as the most potent analogue with a GI_{50} of 21.0 µM. Although less active



Reagents and conditions: (i) NaOH, Ethanol, Reflux 7-8 h, (ii) NH_2-NH_2 .H_2O, Ethanol, Reflux 7-8 h.

Scheme 1 — Reaction scheme for the preparation of compounds **4a-l**

Table 1 — *In vitro* anticancer screening of imidazo[1,2-a]pyridine-thiazole hybrid pyrazolines **4a-l** against human breast cancer cell line MCF-7[a]



Compd	R_1	R_2	R_3	MCF-7 LC_{50}[b]	MCF-7 TGI[c]	MCF-7 GI_{50}[d]
4a	-H	-F	-H	>100	>100	>100
4b	-Cl	-F	-H	>100	>100	>100
4c	$-OCH_3$	-F	-H	>100	>100	>100
4d	-H	$-CH_3$	-H	>100	>100	>100
4e	-Cl	$-CH_3$	-H	>100	>100	>100
4f	$-OCH_3$	$-CH_3$	-H	>100	>100	>100
4g	-H	-Cl	$-CH_3$	>100	>100	>100
4h	-Cl	-Cl	$-CH_3$	>100	>100	>100
4i	$-OCH_3$	-Cl	$-CH_3$	>100	>100	>100
4j	-H	-CN	$-CH_3$	>100	>100	54.0
4k	-Cl	-CN	$-CH_3$	>100	>100	21.0
4l	$-OCH_3$	-CN	$-CH_3$	>100	>100	>100
ADR	–	–	–	>100	10.0	0.4

[a] Concentrations in μM; [b] Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) calculated from [(*Ti* - *Tz*)/*Tz*]x100 = -50; [c] Drug concentration resulting in total growth inhibition (TGI) will calculated from *Ti* = *Tz*; [d] Growth inhibition of 50% (GI_{50}) calculated from [(*Ti* - *Tz*)/(*C* - *Tz*)] x 100 = 50.

than the reference drug Adriamycin (GI_{50} = 0.4 μM), compound **4k** demonstrates a notable enhancement in activity compared with the rest of the series, highlighting the positive influence of the cyano substituent on anticancer potential. Furthermore, comparison of the TGI (total growth inhibition) and LC_{50} (lethal concentration for 50% cell death) values with those of Adriamycin indicated that all synthesized compounds **(4a–l)** were inactive in these measures, as both TGI and LC_{50} values exceeded 100 μM.

The structure-activity relationship (SAR) analysis of the imidazo[1,2-a]pyridine-thiazole hybrid pyrazolines **(4a-l)** revealed that both the electronic nature and position of substituents on the thiazole and imidazo[1,2-a]pyridine rings substantially influence their anticancer activity against MCF-7 cells. In particular, a *para*-chloro group on the thiazole-linked phenyl ring significantly enhanced cytotoxicity, while the incorporation of a cyano (-CN) group on the imidazo[1,2-a]pyridine moiety further improved potency. These electron-withdrawing substituents appear to favorably modulate the electronic environment and steric profile of the framework, promoting better molecular interaction with the cellular target. Among all derivatives, compound 4k, bearing both *para*-chloro and cyano groups, exhibited the highest activity (GI_{50} = 21.0 μM), highlighting the synergistic contribution of these substituents and providing useful insights for future optimization.

Antimicrobial activity

The synthesized novel imidazo[1,2-a]pyridine-thiazole hybrid pyrazolines **(4a-l)** were evaluated for their *in vitro* antimicrobial activity using the disc diffusion method, as reported in the literature. The antimicrobial efficacy was determined by measuring the diameter of the zone of inhibition (mm). The compounds were tested against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). Tetracycline was employed as the reference standard for antibacterial assays, while fluconazole served as the reference standard for antifungal evaluations. Dimethyl sulfoxide (DMSO) was used as the solvent control, and the results are summarized in Table 2.

All the synthesized compounds exhibited moderate to significant antibacterial activity against Gram-positive strains. In the case of *B. subtilis*, compounds

Table 2 — Antimicrobial activityof imidazo[1,2-a]pyridine-thiazole hybrid pyrazolines **4a-l**

Compd	Zone of inhibition in mm												
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**	Standard
B. subtilis	24	26	21	20	24	26	25	28	16	27	25	32	35
S. aureus	24	20	18	27	26	25	25	28	27	28	29	32	34
E. coli	12	14	14	12	10	13	12	10	9	12	11	12	14
P. aerugenosa	19	12	13	18	16	18	13	14	13	22	21	19	24
C. albicans	12	15	14	17	14	14	13	15	14	12	15	16	34

4b, **4f**, **4h**, **4j**, and **4l** displayed the highest inhibition zones (26–32 mm), indicating enhanced potency. Notably, compound **4l** exhibited the strongest activity (32 mm), approaching the efficacy of tetracycline (35 mm). A similar trend was observed against *S. aureus*, where compounds **4h**, **4j**, **4k**, and particularly **4l** (32 mm) demonstrated substantial inhibitory effects. The consistently superior performance of **4l** across both Gram-positive strains suggests that its structural features may favor stronger interactions with Gram-positive bacterial membranes or intracellular targets.

The activity against Gram-negative pathogens was comparatively lower, which is consistent with the known intrinsic resistance mechanisms of Gram-negative bacteria due to their outer membrane barrier. Among the tested derivatives, compounds **4b**, **4c**, and **4f** showed relatively better activity against *E. coli*, producing inhibition zones of 13–14 mm, which are slightly lower than the standard (14 mm). In contrast, most compounds displayed moderate inhibition against *P. aeruginosa*, with **4j** (22 mm) and **4k** (21 mm) demonstrating the highest activity, suggesting that electron-withdrawing substituents or sterically optimized groups may enhance penetration through Gram-negative cell walls.

All synthesized compounds exhibited mild to moderate antifungal activity against *C. albicans*. Compound **4d** (17 mm) and **4l** (16 mm) demonstrated the highest inhibition within the series; however, the antifungal activities were still significantly lower than fluconazole (34 mm). These results indicate that although the hybrid pyrazoline scaffold offers some degree of antifungal effect, additional structural modifications may be required to achieve potency comparable to standard antifungal agents.

Overall, the imidazo[1,2-a]pyridine-thiazole hybrid pyrazolines exhibited broad-spectrum antimicrobial activity, with several compounds demonstrating strong efficacy against Gram-positive bacteria (Fig. 1). Compound **4l** emerged as the most promising

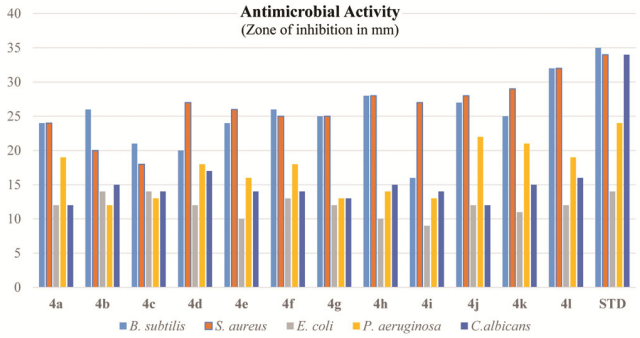


Fig. 1 — Comparison of antimicrobial activityof imidazo[1,2-a]pyridine-thiazole hybrid pyrazolines **4a-l**

candidate, exhibiting consistently high activity across all tested Gram-positive strains and moderate activity against Gram-negative and fungal pathogens. The observed trends underscore the importance of strategic electronic and steric modifications in enhancing antimicrobial potency. These findings suggest that the synthesized hybrid framework holds significant potential for further optimization and development as antimicrobial agents.

Conclusion

In summary, a novel series of imidazo[1,2-a]pyridine–thiazole hybrid pyrazolines **(4a–l)** was successfully synthesized and structurally characterized. Biological evaluation revealed that these compounds possess selective anticancer activity against MCF-7 breast cancer cells, with cyano- and *para*-chloro-substituted derivative **4k** showing the highest potency (GI_{50} = 21.0 µM), highlighting the importance of electron-withdrawing substituents for enhanced cytotoxicity. Additionally, the compounds demonstrated broad-spectrum antimicrobial activity, particularly against Gram-positive bacteria, with compound **4l** emerging as the most promising candidate. The combined anticancer and antimicrobial profiles underscore the potential of this hybrid scaffold for further structural

optimization and development as multifunctional therapeutic agents.

Supplementary Information

Supplementary information is available in the website https://nopr.niscpr.res.in/handle/123456789/58776.

Acknowledgements

The authors sincerely thank Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai 410 210, for performing the *in vitro* anticancer screening.

References

1 Zugazagoitia J, Guedes C, Ponce S, Ferrer I, Molina-Pinelo S & Paz-Ares L, *Clin Ther,* 38 (2016)1551.
2 Sung H , Ferlay J, Siegel R, Laversanne M,Soerjomataram I, Jemal A & Bray F, *CA-Cancer J Clin,* 71 (2021) 209.
3 Bray F, Laversanne M, Sung H, Ferlay J, Siegel R, Soerjomataram I & Jemal A, *CA-Cancer J Clin*, 74 (2024) 229.
4 Harbeck N, Llorca F P, Cortes J, Gnant M, HoussamiN, Poortmans P,Ruddy K, Tsang J & Cardoso F, *Nat Rev Dis Primers*, 5 (2019) 66.
5 Bray F, Ferlay J, Soerjomataram I, Siegel R L, Torre L A & Jemal A, *CA-Cancer J Clin,* 68 (2018) 394.
6 Deep A, Bhatia R K, Kaur R, Kumar S, Jain U K, Singh H, Batra S, Kaushik D & Deb P K, *Curr Top Med Chem*, 17(2017) 238.
7 Narayan A, Patel S, Baile S B, Jain S & Sharma S, *Infect Disord Drug Targets*, 24 (2024) e200324228067.
8 Samanta S, Kumar S, Aratikatla E K, Ghorpade S R & Singh V, *RSC Med Chem*, 14 (2023) 644.
9 Farghaly T A, Alfaifi G H & Gomha S M, *Mini Rev Med Chem*, 24 (2024) 196.
10 Tratrat C, Haroun M, Xenikakis I, Liaras K, Tsolaki E, Eleftheriou P, Petrou A, Aldhubiab B, Attimarad M, Venugopala K N, Harsha S, Elsewedy H S, Geronikaki A & Soković M, *Curr Top Med Chem,* 19 (2019) 356.
11 Guerrero-Pepinosa N Y, Cardona-Trujillo M C, Garzón-Castaño S C, Veloza L A & Sepúlveda-Arias J C, *Biomed Pharm,* 138 (2021) 111495.
12 Shaaban M R, Mayhoub A S & Farag A M, *Expert Opin Ther Pat*, 22 (2012) 253.
13 Ghoneim M M, Abdelgawad M A, Elkanzi N A A & Bakr R B, *Mol Divers*, 29 (2025) 1789.
14 Mantzanidou M, Pontiki E & Hadjipavlou-Litina D*, Molecules*, *26 (2021) 3439.*
15 Ahsan M J, Amena A, Abuzer A, Thiriveedhi A, Bakht M A, Yusuf M, Salahuddin Afzal O & Alfawaz A S A*, ACS Omega,* 7 (2022) 38207.
16 Shringare S, Bhale P, Chavan H, Hundekari P & Kulkarni M, *Croat Chem Acta*, 94 (2021) 191.
17 Dongare S, Chavan H, Surwase D, Bhale P, Mule Y & Bandgar B, *J Chin Chem Soc*, 63 (2016) 323.
18 Bhale P, Chavan H, Shringare S, Khedkar V, Tigote R, Mali N, Jadhav T, Kamble N, Kolat S, Bandgar B & Patil H, *Synth Comm*, 52 (2022) 733.
19 Shringare S, Chavan H, Kamble N, Tigote R, Bhale P, Mali M, Kadam S, Kadam K, Pandhare G, Khalifa A, Pendpale N, Kulkarni M & Bandgar B, *Poly Aromat Comp,* 44 (2024) 5983.
20 Bhale P, Chavan H, Dongare S, Shringare S, Mule Y, Choudhari P & Bandgar B, *Curr Bioact Comp,* 14 (2018) 299.
21 Bhale P, Chavan H, Dongare S, Shringare S, Mule Y, Nagane S & Bandgar B, *Bioorg Med Chem Lett,* 27 (2017) 1502.
22 Dongare S, Bandgar B, Bhale P, Shringare S & Chavan H, *Croat Chem Acta,* 92 (2019) 1-9.
23 Bhale P, Chavan H, Dongare S, Sankpal S & Bandgar B, *Anti-Cancer Agents Med Chem*, 18 (2018) 757.
24 Bhale P, Shringare S, Khade A & Chavan H, *Anti-Cancer Agents Med Chem*, 21 (2021) 2216.
25 Bhale P, Chavan H, Endait R, Kadam A, Bopalkar R, Gaikwad M, *Croat Chem Acta,* 94 (2021) 35.
26 Sirsat D, Bhale P, Chavan H, Karape S & Bachute M, *Rasayan J Chem*, 13 (2020) 1589.
27 Alharbi H, Alsalmi O, Alalawy A I, Qarah A F, Alsimaree A A, Alqahtani A M, Alsoliemy A, El-Metwaly N M, *J Saudi Chem Soc,* 28 (2024) 101800.
28 James P N & Snyder H R, *Org Synth*, 4 (1963) 539.
29 Skehan P, Strong R, Scadiaro D, Monks A, Mc-Mahon J, Vistica D, Warren J, Bokesch H, Kenney S, Boyed M, *J Nat Can Inst,* 82 (1990) 1107.
30 Sri Ramya P, Guntuku L, Angapelly S, Digwal C, Lakshmi U, Sigalapalli D, Babu B, Naidu V & Kamal A, *Eur J Med Chem*, 143 (2018) 216.