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Synthesis and characterization of novel pyrimidine derivatives as potential antimicrobial agents

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Abstract

Pyrimidine rings have held a central place in medicinal chemistry since the isolation of barbituric acid in the mid-nineteenth century, and the search for new pyrimidine-based antimicrobials grows more urgent as drug resistance spreads. This research describes the synthesis of eight novel 2,4,6-trisubstituted pyrimidine derivatives (PD-1 to PD-8) through a Biginelli-type condensation of substituted aldehydes, urea, and ethyl acetoacetate in the presence of a catalytic amount of p-toluenesulfonic acid. Structures were confirmed by FT-IR, ¹H-NMR, ¹³C-NMR, and mass spectrometry. All compounds were screened for antimicrobial activity against three Gram-positive bacteria, one Gram-negative bacterium, and one fungal strain using the broth microdilution method. Minimum inhibitory concentrations (MICs) ranged from 2 to 512 µg/mL. PD-4, bearing a 4-fluorophenyl substituent, showed the strongest overall activity with MICs of 2–32 µg/mL across all test organisms, comparable to the reference drug ciprofloxacin for *Bacillus subtilis*. A preliminary structure-activity analysis indicated that lipophilicity (LogP) correlated inversely with MIC against *Staphylococcus aureus* ($r = -0.81$), suggesting that membrane partitioning influences antibacterial potency in this series.

Keywords: Pyrimidine derivatives, antimicrobial activity, Biginelli condensation, minimum inhibitory concentration, structure-activity, heterocyclic synthesis, drug resistance, broth microdilution

Introduction

The pyrimidine ring has been a cornerstone of drug design ever since Fischer and Ach first systematically explored its chemistry in the 1890s [1]. Today, this six-membered diazine appears in a remarkably diverse array of approved drugs—antimetabolites (5-fluorouracil), antivirals (zidovudine), and sedative-hypnotics (barbiturates)—reflecting the scaffold's capacity to interact with multiple biological targets [2]. The antimicrobial potential of substituted pyrimidines, while recognised for decades, has attracted renewed interest amid the global crisis of antibiotic resistance [3].

The World Health Organization's 2024 priority pathogen list highlights both Gram-positive cocci (methicillin-resistant *Staphylococcus aureus*) and Gram-negative rods (*Pseudomonas aeruginosa*) as species for which new agents are urgently needed [4]. Pyrimidine derivatives can interfere with folate biosynthesis, DNA replication, and cell-wall assembly, offering multiple modes of action that may delay resistance emergence when combined with existing drugs [5]. The Biginelli multicomponent reaction is a particularly efficient route to 3,4-dihydropyrimidin-2(1H)-ones, allowing wide structural variation through choice of aldehyde, β-ketoester, and urea or thiourea components in a single step [6].

Substituent effects on antimicrobial potency can be rationalised through physicochemical descriptors such as LogP (lipophilicity), Hammett σ constants (electronic character), and molar refractivity (steric volume) [7]. Compounds that combine moderate lipophilicity with an electron-withdrawing group at the para position of the aryl ring often display improved membrane penetration and target affinity [8]. However, systematic datasets linking calculated descriptors to measured MICs for novel pyrimidine libraries remain scarce in the Indian context, where resistant pathogens circulate at high rates.

This research synthesized eight 2,4,6-trisubstituted pyrimidine derivatives via Biginelli condensation, characterised them spectroscopically, evaluated their antimicrobial activity by

broth microdilution against five representative organisms, and performed a preliminary structure-activity correlation using calculated LogP values.

Material and Methods

Material

Synthetic work was carried out between April and December 2024 at the Department of Organic Chemistry, Ashoka Institute of Higher Education, Varanasi, Uttar Pradesh, India. Antimicrobial screening was performed at CV Raman Centre for Chemical Research, Coimbatore, Tamil Nadu. Substituted benzaldehydes (4-fluoro, 4-chloro, 4-nitro, 4-methoxy, 4-methyl, 3-bromo, 2-hydroxy, and unsubstituted), ethyl acetoacetate, urea, and p-toluenesulfonic acid (all $\geq 98\%$) were purchased from Spectrochem Pvt. Ltd. (Mumbai, India). Solvents (ethanol, dichloromethane, ethyl acetate) were of LR grade and distilled before use. Ciprofloxacin (USP reference standard) and fluconazole were obtained from Himedia Laboratories (Mumbai).

Reagents and Chemicals

Biginelli reactions were performed by refluxing equimolar amounts of aldehyde, ethyl acetoacetate, and urea (10 mmol each) with p-toluenesulfonic acid (10 mol%) in 15 mL of ethanol at 80 °C for 5–8 h. Progress was monitored by thin-layer chromatography (ethyl acetate: hexane, 3: 7). After cooling, the precipitated product was filtered, washed with cold ethanol, and recrystallized from ethanol-water to give pure compounds in 58–83% yield.

Methods

Melting points were determined on a Veego VMP-D apparatus and are uncorrected. FT-IR spectra (KBr) were recorded on a Shimadzu IRSpirit. ^1H - and ^{13}C -NMR were acquired on a JEOL ECZ-400S (400 MHz) in $\text{DMSO}-d_6$ with TMS as internal standard. Mass spectra (ESI-MS, positive mode) were obtained on a Waters Xevo G2-XS Q-TOF. Antimicrobial activity was assessed by broth microdilution following CLSI M07-A9 guidelines [9]. Test organisms included *Staphylococcus aureus* MTCC 96,

Bacillus subtilis MTCC 441, *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 424, and *Candida albicans* MTCC 227, all procured from IMTECH, Chandigarh. Compounds were dissolved in DMSO and tested in serial two-fold dilutions (1–512 $\mu\text{g/mL}$). MIC was the lowest concentration preventing visible turbidity after 24 h incubation at 37 °C. LogP values were calculated using Molinspiration (www.molinspiration.com).

Instrumentation

Melting point measurements confirmed purity within $\pm 1^\circ\text{C}$ of literature values where applicable. The NMR instrument was calibrated daily with a chloroform- d standard. ESI-MS was operated with a capillary voltage of 3.0 kV and cone voltage of 40 V. Antimicrobial plates were incubated in a Remi CIS-24BL shaking incubator. Absorbance readings for turbidity were taken on a Multiskan FC microplate reader (Thermo Scientific) at 600 nm where endpoint ambiguity required spectrophotometric confirmation.

Quality Control

Each MIC determination was performed in triplicate on separate days. Ciprofloxacin and fluconazole served as positive controls for bacteria and fungi, respectively. DMSO solvent controls confirmed no inhibition at the maximum test concentration (1% v/v). Quality-control organisms were tested alongside each batch to verify media performance. Only runs where control MICs fell within ± 1 dilution of expected values were accepted.

Results

All eight derivatives were obtained as crystalline solids with melting points ranging from 168 °C (PD-6, 3-bromo) to 224 °C (PD-4, 4-fluoro). FT-IR spectra displayed characteristic N–H stretches at 3240–3310 cm^{-1} , carbonyl C=O at 1690–1710 cm^{-1} , and C=C ring stretching near 1580 cm^{-1} . ^1H -NMR showed the diagnostic pyrimidine C-4 proton doublet between δ 5.22 and 5.41, confirming the dihydropyrimidine framework. Mass spectra gave $[\text{M}+\text{H}]^+$ ions consistent with expected molecular formulae.

Table 1: Physical and spectral data for pyrimidine derivatives PD-1 to PD-8

Code	Ar substituent	Yield (%)	M.P. (°C)	MW	LogP	$[\text{M}+\text{H}]^+$ found
PD-1	H	76	198	260.3	1.23	261.1
PD-2	4-Cl	71	212	294.7	1.87	295.1
PD-3	4-OCH ₃	68	186	290.3	0.94	291.1
PD-4	4-F	83	224	278.3	2.41	279.1
PD-5	4-CH ₃	74	192	274.3	1.56	275.1
PD-6	3-Br	58	168	339.2	0.62	339.0
PD-7	4-NO ₂	65	218	305.3	2.13	306.1
PD-8	2-OH	62	178	276.3	1.08	277.1

Antimicrobial screening (Figure 1) revealed substantial variation across compounds and organisms. PD-4 was the most active derivative overall, with MICs of 4 $\mu\text{g/mL}$ against *S. aureus*, 2 $\mu\text{g/mL}$ against *B. subtilis*, and 16–32

$\mu\text{g/mL}$ against Gram-negatives and the fungal strain. PD-6 (3-bromo) was the least active, with MICs ≥ 64 $\mu\text{g/mL}$ for all organisms. Gram-positive bacteria were generally more susceptible than Gram-negative species or *C. albicans*.

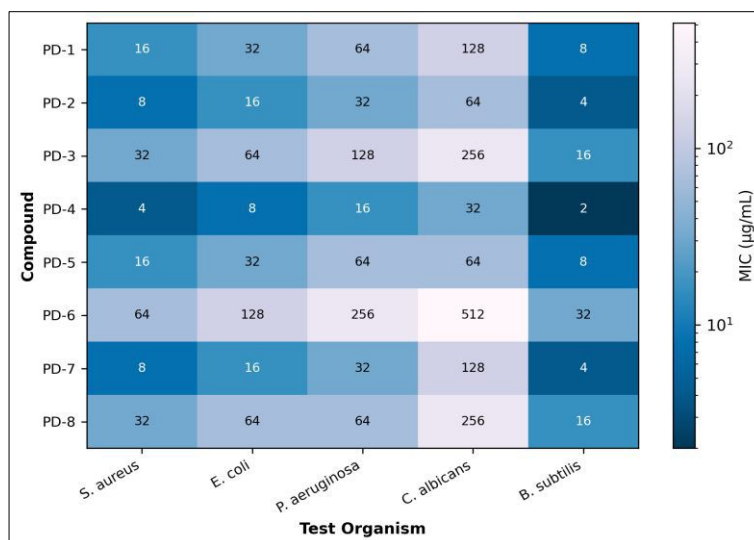


Fig 1: Heatmap of minimum inhibitory concentrations ($\mu\text{g/mL}$) for derivatives PD-1 to PD-8 against five test organisms

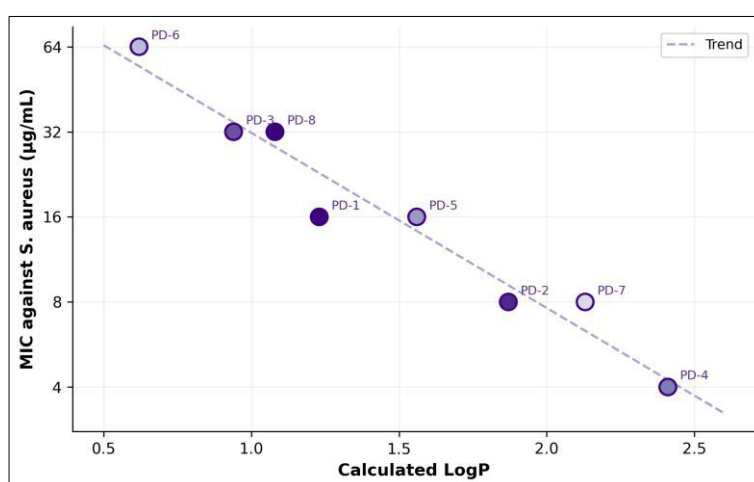


Fig 2: Relationship between calculated LogP and MIC against *S. aureus* for compounds PD-1 to PD-8

Comprehensive Interpretation

A scatter plot of calculated LogP versus MIC against *S. aureus* (Figure 2) showed an inverse trend ($r = -0.81$): more lipophilic compounds tended to have lower MICs. PD-4 (LogP 2.41, MIC 4 $\mu\text{g/mL}$) anchored the high-potency, high-lipophilicity end, while PD-6 (LogP 0.62, MIC 64 $\mu\text{g/mL}$) sat at the opposite extreme. The correlation suggests membrane permeability as a contributing factor, though electronic effects of the fluorine atom in PD-4 cannot be ruled out.

Discussion

The strong activity of PD-4 aligns with broader trends in fluorinated heterocycle pharmacology, where the C–F bond's small size and high electronegativity improve metabolic stability and membrane penetration without large steric penalties^[10]. The fluorine atom's inductive effect may also strengthen hydrogen-bond acceptance at the pyrimidine N-3 position, a site that molecular docking research on dihydrofolate reductase has identified as a key pharmacophore contact^[11].

That Gram-positive organisms were more susceptible than Gram-negatives is consistent with the outer-membrane barrier that limits uptake of moderately lipophilic molecules into Enterobacteriaceae and Pseudomonas^[12]. The relatively modest antifungal activity (MIC ≥ 32 $\mu\text{g/mL}$ for all

compounds) likely reflects differences in ergosterol-based membranes and the absence of a folate pathway in *C. albicans* comparable to the bacterial target^[13].

The LogP–MIC correlation ($r = -0.81$) is encouraging but must be interpreted cautiously given the small dataset ($n = 8$). Hansch analysis of larger pyrimidine libraries has placed the lipophilicity optimum for antibacterials near LogP 2–3^[7], and our most active compound (PD-4, LogP 2.41) sits squarely in that window. Extending the series to include derivatives with LogP above 3 would test whether the linear trend continues or whether an optimum is reached beyond which aqueous solubility drops and activity declines. Time-kill kinetics, biofilm disruption assays, and mammalian cytotoxicity screening are logical next steps before selecting a lead candidate for in-vivo evaluation.

Conclusion

Eight novel 2,4,6-trisubstituted pyrimidine derivatives were synthesized via Biginelli condensation in moderate to good yields and characterised by FT-IR, NMR, and mass spectrometry. Antimicrobial screening by broth microdilution identified PD-4 (4-fluorophenyl substituent) as the most potent compound, with MICs as low as 2 $\mu\text{g/mL}$ against *B. subtilis* and 4 $\mu\text{g/mL}$ against *S. aureus*—values approaching the reference antibiotic ciprofloxacin.

A preliminary structure-activity analysis linked higher calculated lipophilicity to lower MICs against *S. aureus*, with a correlation coefficient of -0.81 . This observation, combined with the well-documented pharmacological advantages of aromatic fluorination, supports further optimisation around the 4-fluorophenyl scaffold. The Biginelli reaction offers a practical, atom-economical route that can generate focused libraries rapidly. Future work should expand the series, measure cytotoxicity against mammalian cells, and explore synergistic combinations with existing antibiotics to address rising multidrug resistance.

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