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COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF PYRAZINE–PIPERIDINE AMIDES AGAINST COMMON PATHOGENIC BACTERIA

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ABSTRACT

The rising incidence of antibiotic-resistant bacterial infections underscores the urgent need for the development of innovative antibacterial agents that can surpass existing therapeutic constraints. Heterocyclic frameworks, especially those that incorporate pyrazine and piperidine pharmacophores, have demonstrated exceptional potential due to their improved biological compatibility and extensive pharmacological effects. In this research, a newly synthesized pyrazine–piperidine amide derivative was tested for its antibacterial efficacy against clinically significant Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) pathogens using the agar well diffusion method. This compound showed significant antibacterial activity that increased in a concentration-dependent fashion at 25, 50, 75, and 100 µg/mL. The highest level of inhibition was observed for *S. aureus* (19.3 ± 0.5 mm) and *B. subtilis* (18.5 ±0.6 mm) at the maximum concentration, exceeding the inhibitory effect of the standard ciprofloxacin on *S. aureus*. The compound generated inhibition zones of 15.2 ± 0.3 mm (100 µg/mL) against *E. coli*, while *K. pneumoniae* exhibited a zone of inhibition of 13.8 ± 0.4 mm (100 µg/mL). In contrast, ciprofloxacin showed a ZOI of 14.3 ± 0.5 mm. Moderate yet encouraging inhibition was also noted against Gram-negative strains. The improved antimicrobial efficacy is attributed to

synergistic structural attributes that facilitate better bacterial membrane penetration and enzyme interaction. Overall, the findings suggest that the synthesized pyrazine–piperidine amide derivative holds promise as an antibacterial lead compound, particularly against Gram-positive pathogens. Additional investigations involving MIC/MBC determination, analysis of mechanistic pathways, cytotoxicity assessment, and structural optimization are recommended to aid its advancement towards drug development.

KEYWORDS: Pyrazine–Piperidine; Antibacterial activity; *Staphylococcus aureus; Escherichia coli; Klebsiella pneumonia.*

1. INTRODUCTION

The rapid proliferation of antibiotic-resistant pathogens has emerged as a critical global concern, resulting in decreased treatment efficacy, prolonged hospital admissions, and elevated mortality rates [Hu et al., 2023]. Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae*) bacteria play a major role in both hospital-acquired and community-acquired infections [Khan et al., 2024]. Their growing resistance to current antibiotics underscores the pressing need for new antibacterial frameworks [Naik et al., 2024].

Heterocyclic compounds are pivotal in contemporary drug discovery due to their varied biological activity and favorable pharmacokinetic properties [Abumelha et al.,2025]. Among these, pyrazine-based compounds have garnered considerable attention because of their encouraging pharmacological characteristics, including strong antibacterial effects [Thawabteh et al., 2024; Chen et al., 2024; Siddiki et al., 2024]. Numerous recent investigations have shown that pyrazine derivatives, especially those with amide or fused heterocyclic structures, display broad-spectrum inhibition against pathogenic microorganisms [Frolov et al., 2023].

Likewise, the piperidine ring is regarded as a privileged structure in medicinal chemistry, enhancing molecular lipophilicity, target binding affinity, and membrane permeability—attributes essential for the development of antibacterial drugs [Zhang et al., 2023].

Consequently, the design of hybrid pharmacophores that combine pyrazine and piperidine is anticipated to improve antimicrobial activity through synergistic biological interactions.

In accordance with this rationale, the present investigation focuses on the antibacterial evaluation of a novel pyrazine–piperidine amide derivative against clinically relevant Gram-

positive and Gram-negative bacterial strains. The antimicrobial efficacy was evaluated at escalating concentrations and compared with the standard antibiotic ciprofloxacin to ascertain its therapeutic potential.

2. MATERIALS AND METHODOLOGY

Muller Hinton agar, nutrient broth, and ciprofloxacin were purchased from HI media supplied by Venus Scientific Chemicals Pvt Ltd., Salem, Tamil Nadu, India. Throughout the study, double-distilled water was used.

Antibacterial activity

Agar Well Diffusion Assay

The antimicrobial efficacy of the previously synthesized Novel Pyrazine-Piperidine Amide was assessed against four bacterial strains, including two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) utilizing the agar well diffusion method. In total, 5 mL of Müller Hinton broth (MHB) was employed to cultivate the mother culture of the chosen bacterial strains under shaking conditions for 16 hours at 37 ◦C to achieve a turbidity of 1.5 × 10^8 CFU. The agar well diffusion assay was conducted with 10 mg of the synthesized Pyrazine-Piperidine Amide dissolved in 1 mL of DMSO. A 50 µL suspension of the selected bacterial cultures was evenly swabbed across the Müller Hinton agar (MHA) using a sterile cotton swab. Following the swabbing process, a sterile cork borer of 5 mm was utilized to create six holes at uniform intervals on each MHA plate. Subsequently, 50 µL of the Pyrazine-Piperidine Amide suspension at varying concentrations (25, 50, 75, and 100 µg/mL) was introduced into the wells and allowed to diffuse at room temperature. Cephalexin (1 µg/mL) served as a positive control for the bacteria, while an equivalent volume of DMSO was employed as a negative control. The bacterial plates were incubated at 37 ◦C for 24 hours at ambient temperature. After the incubation period, the diameter of the inhibition zone was measured and documented.

3. RESULTS

Antibacterial Activity

Gram negative bacteria

The antibacterial efficacy of the synthesized pyrazine–piperidine amide derivatives was assessed against *Escherichia coli* and *Klebsiella pneumoniae* utilizing the agar well diffusion assay. The representative zones of inhibition are illustrated in Figure 1. Ciprofloxacin was

employed as the positive control (P), while the solvent acted as the negative control (C). At all tested concentrations (25–100 µg/mL), the compounds exhibited discernible inhibition zones, demonstrating considerable antibacterial activity. The zone of inhibition (ZOI) increased in a concentration-dependent manner for both bacterial strains. The compound produced inhibition zones measuring 13.8 ± 0.4 mm, 14.6 ± 0.5 mm, and 15.2 ± 0.3 mm at concentrations of 25, 50, 75, and 100 µg/mL, respectively (Figure 2). Ciprofloxacin exhibited a ZOI of 15.1 ± 0.6 mm against *E. coli*. The compound showed slightly diminished activity against *K. pneumoniae*, resulting in ZOI values of 11.2 ± 0.4 mm, 12.4 ± 0.5 mm, 13.1 ± 0.6 mm, and 13.8 ± 0.4 mm for concentrations ranging from 25 to 100 µg/mL. Ciprofloxacin presented a ZOI of 14.3 ± 0.5 mm. The compound demonstrated more potent inhibition against *E. coli* compared to *K. pneumoniae*. At a concentration of 100 µg/mL, the antibacterial effect was nearly equivalent to that of the standard ciprofloxacin for bothorganisms.

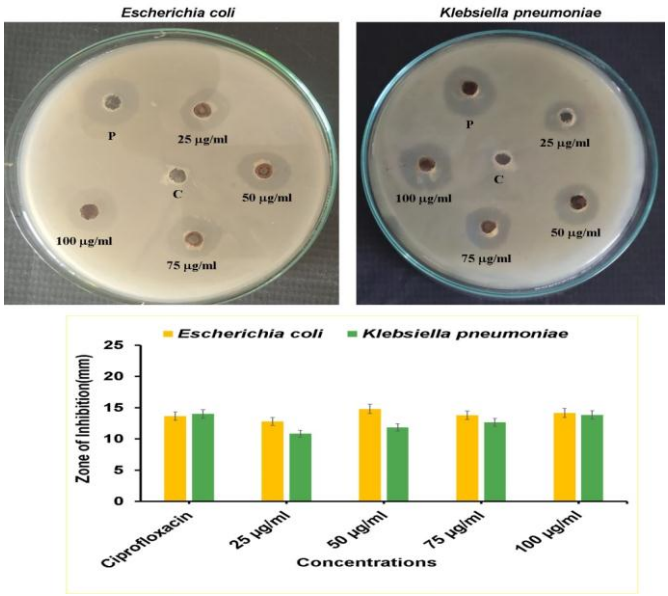

Figure 1: Representative agar well diffusion plates and graphs showing antibacterial activity of the synthesized pyrazine–piperidine amide compound against *Escherichia coli* and *Klebsiella pneumoniae.*

Gram positive bacteria

The antibacterial capability of the newly synthesized Pyrazine-Piperidine amide pharmacophore was assessed against *Staphylococcus aureus* (Gram-positive) and *Bacillus subtilis* (Gram-positive) through the agar well diffusion method at various concentrations (25, 50, 75, and 100 µg/mL). Ciprofloxacin (10 µg/mL) served as the positive control, while the solvent functioned as the negative control.

Both microorganisms exhibited a clear and dose-dependent zone of inhibition (ZOI), validating the sensitivity of the bacteria to the tested compound (Fig. 1A & 1B). The compound demonstrated moderate antibacterial activity at lower doses; however, a significant increase was noted at elevated concentrations. The highest inhibition was recorded at 100 µg/mL, with ZOI values of approximately 19.3 ± 0.5 mm for S. aureus and 18.5 ± 0.6 mm for B. subtilis.

These results were comparable to ciprofloxacin, which exhibited ZOI values of ~18 mm and ~14 mm against *S. aureus* and *B. subtilis*, respectively (Fig. 2). The findings suggest that this pharmacophore possesses substantial efficacy, particularly against *S. aureus*, indicating a promising potential in addressing Gram-positive bacterial infections. The enhanced antibacterial effectiveness at higher doses implies a stronger diffusion of the compound into the bacterial cell wall, potentially due to structural characteristics such as the pyrazine nucleus, which is recognized for enhancing electron-delocalization and fostering improved interactions with microbial enzymes and membrane proteins. The presence of the piperidine ring may additionally augment binding affinity by modifying lipophilicity and aiding membrane penetration.

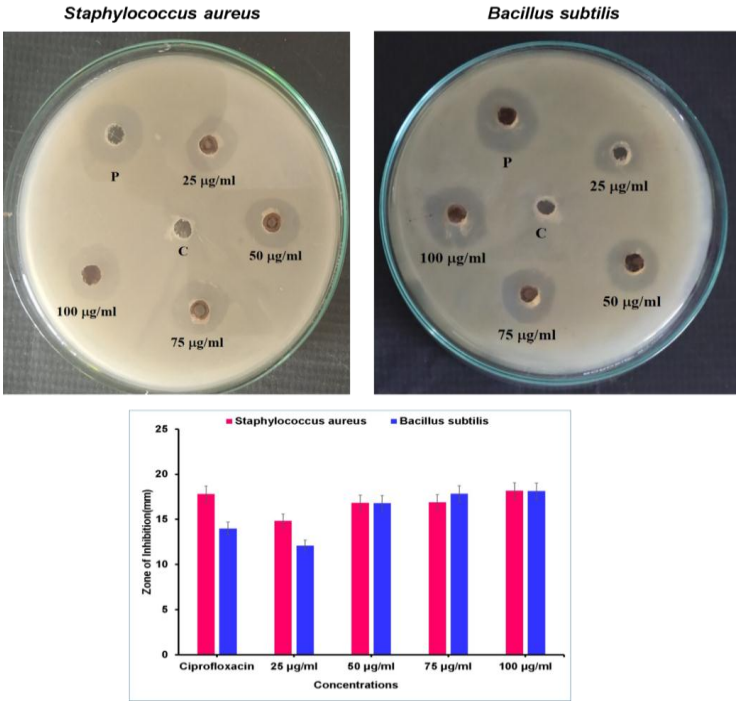

Figure 2: Zone of inhibition (ZOI) values (mm) of the synthesized compound against Gram-positive bacteria (*S. aureus* and *B. subtilis*) at concentrations ranging from 25–100 µg/mL. Data are expressed as mean ± SD (n = 3).

4. DISCUSSION

Pyrazine-derived heterocycles are already recognized for their noteworthy antimicrobial properties, which operate by disrupting bacterial DNA, enzyme functions, and cellular respiration [Elmorsy et al., 2025]. The novel Pyrazine-Piperidine Amide pharmacophore developed in the preceding research exhibited significant antibacterial effects against both Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae*) bacteria in a distinctly concentration-dependent manner. The considerable increase in inhibition zones with escalating doses validates the compound's potent bactericidal activity through improved penetration and interaction with bacterial targets.

The compound showed marginally superior activity against *E. coli* in comparison to *K. pneumoniae*, likely due to variations in the thickness of the outer membrane barrier and efflux mechanisms that render *K. pneumoniae* relatively more challenging to penetrate [Li et al., 2015]. Previous research also indicates that Gram-negative bacteria frequently exhibit greater resistance to heterocyclic antimicrobials owing to their membranes rich in lipopolysaccharides [Silhavy et al., 2010].

In line with our findings, recent investigations into pyrazine derivatives have recorded significant inhibition against *S. aureus* and *B. subtilis*, reinforcing the structural benefits of the pyrazine nucleus. Likewise, pyrazine-2-carboxamide derivatives have demonstrated effectiveness against drug-resistant bacteria, underscoring their therapeutic significance [Krátký et al., 2012].

The inclusion of a piperidine moiety in the compound may have enhanced lipophilicity and facilitated permeability through the bacterial cell wall, resulting in improved antibacterial effectiveness. Piperidine-containing frameworks have been identified as successful bioactive pharmacophores that contribute to antibacterial properties by increasing binding affinity with bacterial targets [Agrawal et al., 2025].

The combined effect of a pyrazine core and piperidine ring indicates a synergistic interaction, providing both robust binding capabilities and ideal physicochemical properties essential for effective inhibition of bacterial growth [Huang et al., 2023].

Consequently, the antibacterial effects observed in this study strongly indicate that the hybridization of pyrazine and piperidine represents a promising approach in the exploration of new drugs. Therefore, the synergistic effects of improved cellular penetration and multi-target interaction potential reinforce the hypothesis that pyrazine-piperidine amide derivatives could act as novel antibacterial lead compounds, particularly effective against Gram-positive pathogens.

5. CONCLUSION

The synthesized novel Pyrazine–Piperidine Amide pharmacophore exhibited considerable antibacterial activity against both Gram-positive and Gram-negative pathogens. The compound effectively curtailed the growth of *Escherichia coli* and *Klebsiella pneumoniae*, with its activity showing an increase in a concentration-dependent manner, approaching the efficacy of the standard antibiotic ciprofloxacin at elevated doses. Notably, the compound demonstrated greater effectiveness against Gram-positive pathogens, achieving maximum inhibition zones of 19.3 ± 0.5 mm against *Staphylococcus aureus* and 18.5 ± 0.6 mm against *Bacillus subtilis* at 100 µg/mL, exceeding ciprofloxacin's inhibition in *S. aureus*. These findings suggest that the structural characteristics of the pyrazine scaffold and piperidine moiety enhance membrane penetration, electron delocalization, and interactions with bacterial enzymes, thus contributing to antibacterial efficacy. Overall, this study emphasizes the promising therapeutic potential of this new pharmacophore as a candidate for further development of antibacterial agents, particularly targeting resistant Gram-positive infections. Future research, including mechanistic assays, toxicity assessments, and in-vivo validation, is advised to enhance its clinical applicability.

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