

Development And Structural Elucidation of Novel Azomethine Derivatives with Potent Microbicidal Profiles

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ABSTRACT: The escalating global health crisis emanating from the emergence and proliferation of multidrug-resistant (MDR) microbial pathogens represents one of the most pressing challenges confronting modern clinical interventions. This study focuses on the design, synthesis, and evaluation of novel azomethine derivatives with potential antimicrobial activity. A series of compounds were synthesized through condensation reactions between substituted aromatic aldehydes and aniline using ethanol as solvent and glacial acetic acid as a catalyst. The synthesized compounds (AZ-1 to AZ-6) were characterized using FT-IR, NMR, and mass spectrometry techniques, confirming the successful formation of the azomethine linkage. Antimicrobial activity was assessed against Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungal (*Candida albicans*) strains. The results demonstrated that compounds containing electron-withdrawing groups exhibited enhanced antimicrobial potency, with dichloro- and nitro-substituted derivatives showing the highest activity (MIC 4–8 µg/mL). Structure–activity relationship analysis revealed that substituent nature significantly influences biological performance.

I. INTRODUCTION

The escalating global health crisis emanating from the emergence of multidrug-resistant (MDR) microbial pathogens represents one of the most pressing challenges confronting modern therapeutics [cite: 26]. Current projections indicate that antimicrobial resistance (AMR) could precipitate approximately 39 million deaths between the present and 2050 if innovative therapeutic strategies fail to materialize [cite: 27]. Pathogenic microorganisms continue to acquire sophisticated resistance mechanisms, including

genetic and biochemical adaptation [cite: 28]. ESKAPE pathogens, such as MRSA and carbapenem-resistant Enterobacteriaceae, exemplify the threat landscape [cite: 28, 50].

Azomethine compounds, characterized by the characteristic imine or azomethine functional group ($-C=N-$), have emerged as a promising avenue within medicinal chemistry [cite: 29]. These compounds are conventionally synthesized through condensation reactions between primary amines and carbonyl substrates [cite: 30]. The polarized $C=N$ double bond engages in non-covalent interactions with biological targets, facilitating potent interactions with cellular components [cite: 34]. This study endeavors to synthesize a novel series of azomethine derivatives through rational scaffold design and comprehensive characterization [cite: 45].

II. LITERATURE REVIEW

Azomethine derivatives, commonly known as Schiff bases, have emerged as an essential class of compounds due to their versatile coordination behavior and significant biological potential [cite: 81]. These compounds have shown remarkable antimicrobial, antifungal, and antiviral properties [cite: 82]. Recent studies highlight that structural modifications, particularly the introduction of heteroatoms and aromatic substituents, significantly enhance bioactivity [cite: 83].

Extensive epidemiological and clinical data confirm that AMR is now a pervasive problem across hospital and community settings [cite: 99]. Conventional strategies based on incremental structural modification of existing antibiotics have reached a bottleneck [cite: 101]. This situation has stimulated interest in alternative chemotypes, such as heterocyclic scaffolds that can be tuned to interact with multiple microbial targets [cite: 54]. Azomethine nitrogen is typically sp^2 -hybridized, and the $C=N$ bond adopts a planar configuration

that promotes crystallinity and bioactivity [cite: 109].

III. RATIONALE AND HYPOTHESIS

The characteristic C=N double bond moiety represents a promising class of heterocyclic ligands with versatile pharmacological potential [cite: 184]. Growing AMR demands innovative therapeutic strategies beyond conventional antibiotics [cite: 185]. Through systematic structure-activity relationship studies, we aim to elucidate the influence of aromatic substitution patterns on antibacterial and antifungal properties [cite: 187]. It is hypothesized that these derivatives will exhibit potent activity by disrupting microbial cell membranes and inhibiting essential enzymes like DNA gyrase [cite: 191].

IV. METHODOLOGY

4.1 Chemicals and Reagents

Key reagents included 4-Chlorobenzaldehyde, 4-Nitrobenzaldehyde, 4-Methoxybenzaldehyde, 3,4-Dichlorobenzaldehyde, 2-Hydroxybenzaldehyde, 4-Fluorobenzaldehyde, and Aniline [cite: 201]. Absolute ethanol was used as the solvent, and glacial acetic acid served as the catalyst [cite: 201].

4.2 Synthesis of Azomethine Derivatives

Novel azomethine derivatives were synthesized via a one-pot condensation reaction [cite: 204]. The reaction proceeded in absolute ethanol, with a catalytic amount of glacial acetic acid added to facilitate imine formation by promoting

nucleophilic attack [cite: 205]. The mixture was refluxed at 80°C for 4-6 hours under an inert nitrogen atmosphere, monitored by TLC [cite: 206]. Pure products were obtained through recrystallization from ethanol with yields of 75-92% [cite: 206].

4.3 Structural Characterization Techniques

Structural confirmation employed a suite of spectroscopic methods [cite: 274]:

- **FT-IR Spectroscopy:** Revealed characteristic C=N stretching vibrations at 1620-1650 cm^{-1} [cite: 275].
- **¹H-NMR Spectroscopy:** Displayed azomethine proton signals at δ 8.2-9.0 ppm as singlets [cite: 276].
- **¹³C-NMR Spectroscopy:** Confirmed C=N carbon at 160-165 ppm [cite: 276].
- **High-Resolution Mass Spectrometry (HRMS):** Provided molecular ion peaks matching calculated m/z values [cite: 274, 277].

V. RESULTS AND DISCUSSION

5.1 Synthesis and Physicochemical Properties

Six azomethine derivatives (AZ-1 to AZ-6) were successfully synthesized [cite: 293]. The reactions proceeded smoothly under reflux conditions, yielding crystalline compounds with high purity [cite: 294].

Table 1: Physicochemical properties and yields of synthesized derivatives [cite: 298]

Code	Aromatic Aldehyde	Mol. Formula	Mol. Wt (g/mol)	Yield (%)	Melting Pt (°C)
AZ-1	4-Cl-Benzaldehyde	C ₁₃ H ₁₀ ClN	215.68	82.4	178–180
AZ-2	4-NO ₂ -Benzaldehyde	C ₁₃ H ₁₀ N ₂ O ₂	226.23	88.1	192–194
AZ-3	4-OCH ₃ -Benzaldehyde	C ₁₄ H ₁₃ NO	211.26	79.3	164–166
AZ-4	3,4-DiCl-Benzaldehyde	C ₁₃ H ₉ Cl ₂ N	250.12	91.2	201–203
AZ-5	2-OH-Benzaldehyde	C ₁₃ H ₁₁ NO	197.23	76.8	171–173
AZ-6	4-F-Benzaldehyde	C ₁₃ H ₁₀ FN	199.22	86.7	184–186

5.2 Antimicrobial Activity Assessment

Antibacterial and antifungal potencies were evaluated using agar well diffusion and broth microdilution assays [cite: 279]. Compounds exhibited variable inhibitory activities depending on the nature of the substituents [cite: 330].

Table 2: Antimicrobial activity (Zone of Inhibition in mm) [cite: 337]

Code	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
AZ-2	24.1 ± 0.7	21.3 ± 0.6	20.2 ± 0.5	22.5 ± 0.4
AZ-4	26.5 ± 0.6	23.9 ± 0.5	22.7 ± 0.6	24.3 ± 0.5
AZ-6	22.8 ± 0.5	20.4 ± 0.6	19.6 ± 0.5	21.1 ± 0.6
Standard	29.4 (Cip)	27.8 (Cip)	26.2 (Cip)	28.1 (Flu)

5.3 Minimum Inhibitory Concentration (MIC)

MIC analysis demonstrated that AZ-4 possessed the highest antimicrobial potency, with values ranging from 4–8 µg/mL [cite: 346]. Nitro-substituted AZ-2 followed with values between 8–16 µg/mL [cite: 346]. The enhanced activity of these derivatives is attributed to electron-withdrawing substituents that increase membrane permeability [cite: 347].

VI. ADVANCED STRUCTURAL ELUCIDATION

X-ray crystallography on single crystals confirmed the E-configuration around the C=N bond and the planarity of the conjugated systems [cite: 283]. AZ-4 exhibited the highest planarity (dihedral angle 5.1°), contributing to its superior biological activity [cite: 363]. DFT calculations optimized geometries and analyzed frontier molecular orbitals, showing HOMO-LUMO gaps of 3.5-4.2 eV indicative of high bioactivity [cite: 284].

VII. CONCLUSION

In conclusion, the study successfully synthesized and characterized a series of novel azomethine derivatives with promising antimicrobial properties [cite: 389]. Biological evaluation demonstrated that derivatives containing electron-withdrawing substituents, particularly dichloro- (AZ-4) and nitro- (AZ-2) groups, exhibited superior antimicrobial activity compared to electron-donating counterparts [cite: 391, 392]. These findings highlight the significance of structural modification in enhancing biological activity and suggest that azomethine derivatives represent a valuable scaffold for next-generation antimicrobial agents [cite: 393, 394].

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