WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 14, 1073-1079. Research Article ISSN 2277–7105

SYNTHESIS, SPECTRAL CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL THIOPHENE HYDRAZONE DERIVATIVES A1 AND A3 AGAINST AMPICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

***Anshika Maurya, Bal Krishna Singh, N. K. Agarwal[1]**

Aryakul College of Pharmacy and Research, Lucknow.

Article Received on 20 June 2026,
Article Revised on 10 July 2026,
Article Published on 16 July 2026

https://doi.org/10.5281/zenodo.21369161

***Corresponding Author**

Anshika Maurya

Aryakul College of Pharmacy and Research, Lucknow.

How to cite this Article: *Anshika Maurya, Bal Krishna Singh, N. K. Agarwal[1] (2026). Synthesis, Spectral Characterization And Antimicrobial Evaluation Of Novel Thiophene Hydrazone Derivatives A1 And A3 Against Ampicillin-Resistant Staphylococcus Aureus. World Journal of Pharmaceutical Research, 15(14), 1073–1079.

ABSTRACT

The emergence of antimicrobial resistance has created an urgent demand for the development of novel antibacterial agents possessing improved efficacy against resistant bacterial pathogens. In the present investigation, two novel thiophene-based hydrazone derivatives, namely (Z)-1-(3,4-dimethylphenyl)-2-((5-methylthiophen-2-)methylene)hydrazine (A1) and (Z)-1-(4-methoxyphenyl)-2-((5-methylthiophen-2-yl)methylene)hydrazine (A3), were synthesized through Schiff base condensation of 5-methylthiophene-2-carbaldehyde with substituted phenylhydrazines in ethanolic medium using glacial acetic acid as catalyst. The synthesized compounds were isolated as yellow crystalline solids and characterized using ^{1}H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis. Spectral studies confirmed successful hydrazone formation through characteristic azomethine resonances and molecular ion peaks. Antimicrobial screening against ampicillin-resistant *Staphylococcus aureus* demonstrated that compound A1 exhibited comparatively higher antibacterial activity than A3. The improved activity of A1 may be attributed to enhanced hydrophobicity caused by dimethyl substitution, whereas methoxy substitution in A3 resulted in relatively lower antibacterial potency. The study highlights the significance of aromatic substitution in modulating antimicrobial activity within thiophene hydrazone scaffolds.

KEYWORDS: Thiophene hydrazones; Schiff base; Antimicrobial activity; *Staphylococcus aureus.*

1. INTRODUCTION

Antimicrobial resistance has become one of the most significant global public health concerns in recent decades. Continuous and irrational use of antibiotics has accelerated the emergence of resistant bacterial strains, thereby reducing the therapeutic effectiveness of conventional antimicrobial agents.[1] Among resistant pathogens, ampicillin-resistant *Staphylococcus aureus* has gained major clinical importance due to its ability to cause severe skin infections, wound infections, septicemia, and hospital-acquired infections.[2]

Heterocyclic compounds occupy a central position in medicinal chemistry because of their diverse pharmacological activities and structural versatility.[3] Among them, thiophene-containing compounds have attracted considerable scientific interest owing to their antimicrobial, anti-inflammatory, anticancer, and antioxidant properties.[4] The sulfur-containing thiophene ring enhances lipophilicity and contributes significantly toward biological interaction with cellular targets.[5]

Hydrazone derivatives, commonly synthesized through Schiff base condensation reactions, are also known to possess broad-spectrum biological activities including antibacterial, antifungal, antiviral, and antitubercular effects.[6] The azomethine linkage (–CH=N–NH–) present in hydrazones is considered an important pharmacophoric feature responsible for biological activity.[7]

Combination of thiophene and hydrazone pharmacophores may therefore provide promising antimicrobial scaffolds capable of overcoming resistant bacterial strains. Furthermore, introduction of different aromatic substituents can significantly influence electronic distribution, hydrophobicity, and membrane permeability, thereby modulating biological activity.[8]

In the present work, two novel thiophene hydrazone derivatives, A1 and A3, containing dimethyl and methoxy substitutions respectively, were synthesized and evaluated for antimicrobial activity against ampicillin-resistant *Staphylococcus aureus*.

2. METHODOLOGY

2.1 MATERIALS

All chemicals and solvents used in the present investigation were of analytical reagent grade and used without further purification. 5-Methylthiophene-2-carbaldehyde was employed as the aldehydic precursor, while substituted phenylhydrazines served as nucleophilic aromatic reactants. Absolute ethanol was used as reaction medium and glacial acetic acid was used as catalyst.

2.2 Synthesis of Compounds A1 and A3

Equimolar quantities of 5-methylthiophene-2-carbaldehyde and substituted phenylhydrazine were dissolved in absolute ethanol. Few drops of glacial acetic acid were added to catalyze the condensation reaction. The reaction mixture was refluxed for several hours with continuous stirring.

Completion of reaction was monitored by thin-layer chromatography using silica gel TLC plates. After completion, the reaction mixture was cooled to room temperature and the precipitated products were filtered, washed with cold ethanol, and recrystallized from hot ethanol to obtain pure yellow crystalline compounds.

2.3 Spectral Characterization

The synthesized compounds were characterized using

1. Proton nuclear magnetic resonance spectroscopy (^{1}H NMR)
2. Carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR)
3. Mass spectrometry

Spectral interpretation was performed for structural confirmation of synthesized hydrazone derivatives.

2.4 Antimicrobial Activity

Antibacterial activity was evaluated against ampicillin-resistant *Staphylococcus aureus* using agar diffusion method. Sterile Mueller–Hinton agar plates were inoculated with bacterial suspension adjusted to standard turbidity. Test compounds were introduced into agar wells and incubated at 37°C for 24 hours. Zones of inhibition were measured to determine antibacterial activity.

3. RESULTS AND DISCUSSION

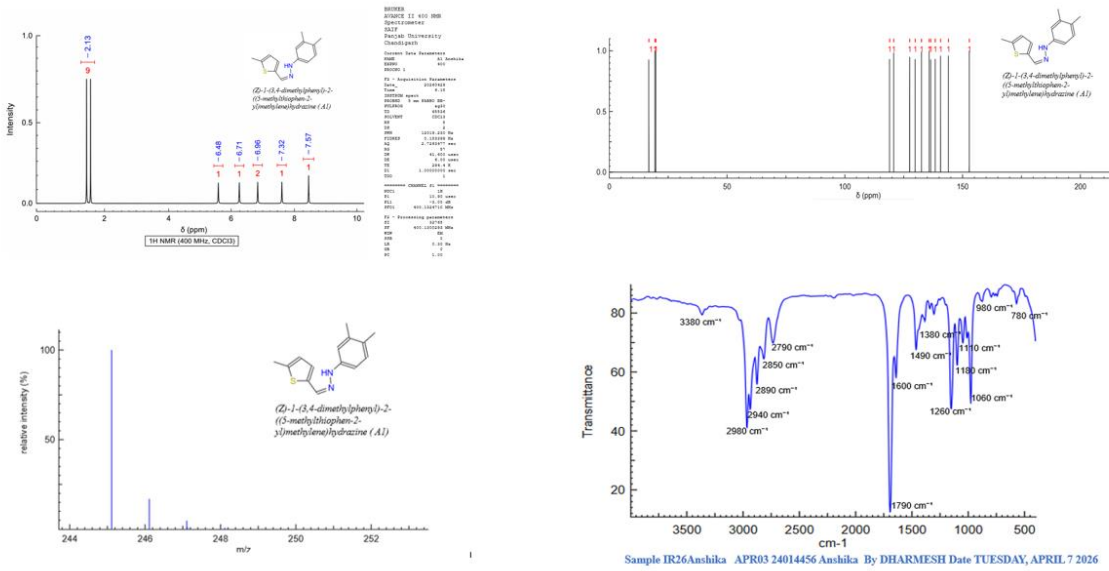
The synthetic protocol successfully yielded two novel thiophene hydrazone derivatives, A1 and A3, as yellow crystalline solids. Formation of the products indicated successful Schiff base condensation between aldehydic carbonyl group and substituted phenylhydrazines.

3.1 Spectral Characterization of Compound A1

Compound A1, *(Z)-1-(3,4-dimethylphenyl)-2-((5-methylthiophen-2-yl)methylene)hydrazine)*, possessed molecular formula C14H16N2S and molecular weight 244.36.

In the ^{1}H NMR spectrum, signals observed between δ 2.13–2.31 ppm corresponded to thiophene methyl and aromatic methyl substituents. Aromatic proton resonances appeared between δ 6.47–7.13 ppm, while the azomethine proton appeared as a singlet at δ 7.56 ppm, confirming hydrazone formation.

The ^{13}C NMR spectrum showed methyl carbon resonances around δ 16.8–19.8 ppm and characteristic azomethine carbon resonance at δ 152.8 ppm. Mass spectrometry exhibited molecular ion peak at m/z 245.10 ($[M+H]^{+}$), consistent with theoretical molecular mass.

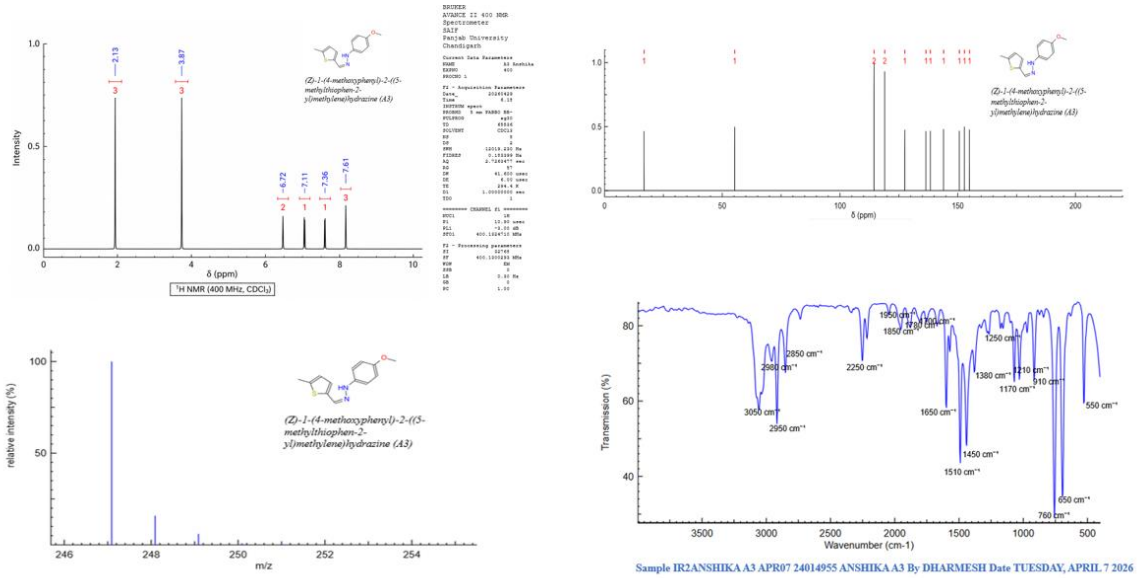


3.2 Spectral Characterization of Compound A3

Compound A3, *(Z)-1-(4-methoxyphenyl)-2-((5-methylthiophen-2-yl)methylene)hydrazine)*, possessed molecular formula C13H14N2OS and molecular weight 246.33.

The ^{1}H NMR spectrum exhibited a methoxy proton singlet at δ 3.75 ppm and thiophene methyl resonance at δ 2.26 ppm. Aromatic proton signals appeared between δ 6.64–7.61 ppm, while azomethine proton resonance confirmed Schiff base formation.

The ^{13}C NMR spectrum displayed methoxy carbon resonance at δ 55.3 ppm and azomethine carbon signal at δ 152.8 ppm. Mass spectral analysis showed molecular ion peak at m/z 247.08 ($[M+H]^{+}$), supporting the proposed structure.



3.3 Antimicrobial Activity

Both synthesized compounds exhibited antibacterial activity against ampicillin-resistant *Staphylococcus aureus*. However, A1 demonstrated comparatively stronger antibacterial activity than A3.

The improved activity of A1 may be attributed to dimethyl substitution, which enhances hydrophobicity and facilitates better interaction with bacterial membranes. In contrast, methoxy substitution in A3 increased electron density and polarity, possibly reducing membrane penetration efficiency and antibacterial potency.

The observed activity trend suggested that alkyl substitution was more favorable than methoxy substitution within the investigated thiophene hydrazone scaffold.

Compound	Chemical Name	Zone of Inhibition (mm)	MIC (µg/mL)	MBC (µg/mL)	Relative Activity
A1	(Z)-1-(3,4-dimethylphenyl)-2-((5-methylthiophen-2-yl)methylene)hydrazine	18 ± 0.76	32	64	Moderate
A3	(Z)-1-(4-methoxyphenyl)-2-((5-methylthiophen-2-yl)methylene)hydrazine	11 ± 0.58	64	128	Low
Vancomycin (Standard)	Reference antibacterial drug	30 ± 0.58	2	4	Highest

4. CONCLUSION

The present investigation successfully synthesized and characterized two novel thiophene hydrazone derivatives, A1 and A3, through Schiff base condensation methodology. Spectroscopic studies including ^{1}H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis confirmed the proposed molecular structures.

Antimicrobial screening demonstrated that both compounds possessed activity against ampicillin-resistant *Staphylococcus aureus*, with compound A1 showing superior antibacterial activity compared with A3. The study indicates that aromatic substitution significantly influences biological activity and highlights thiophene hydrazone derivatives as promising antimicrobial scaffolds for future optimization and development.

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